Summer 8-2017

Taxonomy and Systematics of Plagioporus (Trematoda), With Descriptions of 10 New Species From Freshwater Fishes Of The Nearctic

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TAXONOMY AND SYSTEMATICS OF PLAGIOPORUS (TREMATODA), WITH DESCRIPTIONS OF 10 NEW SPECIES FROM FRESHWATER FISHES OF THE NEARCTIC

by

Thomas John Fayton

A Dissertation
Submitted to the Graduate School,
the College of Science and Technology,
and the School of Ocean Science and Technology
at The University of Southern Mississippi
in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy

August 2017

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ABSTRACT

TAXONOMY AND SYSTEMATICS OF PLAGIOPORUS (TREMATODA), WITH DESCRIPTIONS OF 10 NEW SPECIES FROM FRESHWATER FISHES OF THE NEARCTIC

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The Opecoelidae Ozaki, 1925 is one of the largest families of digenetic trematodes of fishes. While the family is mostly marine/estuarine, invasion of freshwater hosts has occurred at least two times. The only representative freshwater plagioporine sequenced to date is *Plagiocirrus loboides* Curran, Overstreet, & Tkach, 2007, which previous phylogenetic analyses resolved as being related to deep water marine opecoelids. The taxonomy of the freshwater plagioporines, particularly *Plagioporus*, has long been confused; homoplasy is rife within the family and has complicated the delineation of species and genera, and the freshwater species from marine forms. Here, I hypothesize that the freshwater plagioporines, including *Plagioporus*, form a monophyletic group and that intestinal *Plagioporus* have radiated across many families of freshwater/anadromous fish and within several, particularly for the cyprinids, percids and catostomids. I describe 10 new species and 2 new forms of *Plagioporus* from North America, and redescribe 3 congeners. I obtained sequences of the ITS1+2 and 28S rDNA gene regions of these new species and forms, 5 previously described congeners from the Nearctic and also from 5 species of *Neoplagioporus* and *Urorchis* from Japan. Bayesian inference analysis of 28S and 28S concatenated with ITS2 revealed that the freshwater plagioporines form a monophyletic group, with species from the Nearctic resolved as

sister to those from the Palearctic with high support. *Plagiocirrus loboides* was nested within the clade composed of members of *Plagioporus* and was therefore transferred to *Plagioporus*. *Plagioporus* was amended to accommodate a posteriorly extending uterus and restricted vitelline field, two characters that were also shown to be problematic in distinguishing *Urorchis* from *Neoplagioporus*. *Plagioporus* was further amended to accommodate 2 species from Arkansas with long excretory vesicles.

Nearctic *Plagioporus* now comprise a monophyletic group with species from cyprinid, catostomid, percid, salmonid, gasterosteid, fundulid, ictalurid and cottid definitive hosts. In addition, morphological and molecular data suggest that monophyletic radiations of intestinal *Plagioporus* have occurred within the percids, cyprinids and catostomids. With 23 species, *Plagioporus* is now the most diverse digenean genus of fish trematodes in the Nearctic and one of the most successful in terms of its radiation across fish host families.

ACKNOWLEDGMENTS

This work would not have been possible without the support of my parents, who provided financial support and also allowed me to work up my dissertation for 21 months in a makeshift lab in their home in New Hampshire. I am indebted to Dr. Anindo Choudhury (St. Norbert College) for providing lab space, microscopy equipment and molecular supplies while working up species description at St. Norbert College in De Pere, Wisconsin and for providing specimens of *P. boleosomi*, *P. kolipinskii* and *P. shawi*. My residence at St. Norbert College would not have been possible without Vassar College Fellowships that provided funds for the collection of freshwater plagioporines and also living expenses while working up species descriptions in Wisconsin, including an Adolph Sutro Fellowship in 2013/2014, a Nancy Skinner Clark Fellowship in 2014/2015 and an additional Nancy Skinner Clark Fellowship in 2015/2016. I appreciate the support of the US Fish and Wildlife Service in allowing me to finish writing my dissertation while also working as a fish biologist. From the University of Southern Mississippi, I thank Dr. Richard Heard and Dr. Robin Overstreet for provision of microscopy equipment, access to the molecular lab and supplies and for their expertise on digenean parasites, Dr. Michael Andres and Dr. Stephen Curran for their expert opinions on parasites and guidance with molecular work, Jean Jovonovich Alvillar and Janet Wright for their assistance with DNA sequencing reactions, Juanma Carillo for help contacting professors during my time away from Mississippi, and Guillermo Sanchez for help collecting fish in California. I further thank Dr. Peter B. Moyle and his lab members for help collecting fish in California. I am grateful to Dr. Charles Criscione (Texas A&M) for providing specimens of *P. shawi*. I'm also thankful to Sean Locke (University of

Puerto Rico-Mayagüez), David Marcogliese (Environment Canada) and associated personnel from Environment Canada who aided in the collection of fish hosts of *P. sinitsini* and *P. serotinus* and provided lab space for dissections in Montreal, Canada. Moreover, I am appreciative of William F. Font (University of Southern Louisiana) for informing me of the *Plagioporus* sp. from *C. carolinae* in Arkansas. Sampling of *Plagioporus* from the Great Smokey Mountains National Park would not have been possible without the assistance of the National Park Service (NPS), especially Matt Kulp, Rebecca Nichols and Paul Super as well as the provision of a scientific research and collecting permit (#GRSM-2014-SCI-1153). I thank Dr. Sherman S. Hendrix and Dr. Peter Fong (Gettysburg College) for their assistance in collecting fish and snails from Marsh Creek, Pennsylvania, and further am thankful to Dr. Hendrix for informing me of his previous work on fish trematodes in the Great Smokey Mountains. From Virginia Tech, I thank both Dr. Lisa Belden and Sally Zemmer for providing lab space and recommending sampling sites where larval opecoelids had previously been collected in Virginia. I appreciate the help of Dr. James Diana (University of Michigan) for advice on sampling localities and provision of lab space during my attempt to collect *P. lepomis* in Michigan. I also thank Dr. Urabe Misako (University of Shiga Prefecture) and Dr. Takeshi Shimazu for providing their insights on and specimens of opecoelids from Japan. This work would not have been possible without the issuing of scientific permits from various entities, including the Arkansas Game and Fish Commission and the Florida Fish and Wildlife Commission. This project would not have been possible without the sampling assistance of Dr. Chris T. McAllister (Eastern Oklahoma State College) and Matthew B. Connior (Northwest Arkansas Community College) and the fish

identifications of Dr. Henry W. Robison (Southern Arkansas University). Lastly, I deeply appreciate the financial support of the various grants that funded my asssitantships while living in Mississippi, including Dr. Mark Peterson's NOAA-NRDA grant on Atlantic Sturgeon, a NSF museum improvement grant awarded to Dr. Stephen Curran and Dr. Sara Lecroy, a BOEM grant awarded to the fisheries department and Dr. Richard Heard's NRDA grant on decapods. I thank my advisors for taking the time to read and edit this dissertation and being understanding of my unique situation in being a remote student off campus for the past 2 years.

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LIST OF ABBREVIATIONS

USM	The University of Southern Mississippi

CHAPTER I – Introduction

Overview of the Opecoelidae Ozaki, 1925

The Opecoelidae Ozaki, 1925 is one of the largest families of digeneans (a subclass within the Phylum Platyhelminthes, Class Trematoda) of fishes, with a cosmopolitan distribution and consisting of over 800 species in 87 genera (Andres et al., 2014; Bray et al., 2014; Cribb, 2005). The most common definitive hosts are marine, estuarine and freshwater teleosts, though members can reach sexual maturity in invertebrate first or second intermediate hosts or amphibians (Aliff, 1973; Barger & Esch, 2000; Cribb, 2005). Among digeneans, opecoelids are not obviously specialized morphologically and recognition of the family requires a suite of characters, most of which are common in other families. Other families have been historically confused with opecoelids owing to this unremarkable morphology, including the Opistholebetidae Fukui, 1929, Lepocreadiidae Odhner, 1905, Fellodistomidae Nicoll, 1909 and especially, the Allocreadiidae Loss, 1902, to which many opecoelids were originally assigned (Cribb, 2005). A sinistral genital pore (in freshwater forms) as opposed to one that is median or occasionally submedian and the absence of eye-spot pigments scattered in the forebody can be used to distinguish the opecoelids from the allocreadiids. The two families also deviate from one another in their life cycles; opecoelids have sporocysts in prosobranch snails that shed cotylocercous cercariae whereas allocreadiids have rediae mostly in bivalves that shed opthalmoxiphidiocercariae (Cribb, 2005).

While 11 subfamilies have been proposed for the Opecoelidae, most taxonomic studies follow Gibson & Bray (1982; 1984), who recognized four subfamilies, including the Opecoelinae Ozaki, 1925 (type subfamily), Plagioporinae Manter, 1947,

Opecoelininae Gibson & Bray, 1984, and Stenakrinae Yamaguti, 1970, that are primarily distinguished by the form of the male genitalia and sperm reception in the female reproductive system (Cribb, 2005). To date, molecular studies of adult opecoelids have exclusively used rDNA gene sequences (mostly 28S, occasionally 18S) to infer phylogenetic relationships (Andres et al., 2014; Bray et al., 2014, 2016; Shedko et al., 2015). The most recent phylogenetic analyses of the opecoelids, while supporting the monophyly of the family, suggest that the current subfamily level classification is problematic and requires significant revision (Andres et al., 2014; Bray et al., 2014, 2016; Shedko et al., 2015). Olsen et al. (2003) provided the first phylogenetic analysis for the family and found that 2 opistholebetid species nested within the opecoelids within a clade containing plagioporines, a finding that was supported by subsequent phylogenetic analyses (Bray et al., 2014, 2016). Based on the nesting of these opistholebetid species within the Opecoelidae, Bray et al. (2016) relegated the opistholebetids to a subfamily within the Opecoelidae distinguished from others in its unique host association (diodontid and tetraodontid fishes) and morphology. These authors also erected a new subfamily, the Helicometrinae Bray, Cribb, Littlewood, and Waeschenbach, 2016, to accommodate the genera *Helicometra* Odhner, 1902, *Helicometrina* Linton, 1910, and *Neohelicometra* Siddiqi & Cable, 1960 that collectively are unique in the characters of the egg and uterus. The Helicometrinae was found to be resolved as sister to a clade formed by the plagioporines, opecoelinines and opistholebetines (Bray et al., 2016). Andres et al. (2014), Bray et al. (2014, 2016) and Shedko et al. (2015) resolved the Plagioporinae as a polyphyletic group consisting of two clades, within one of which were nested opecoeline species. Bray et al. (2014, 2016) and Shedko et al. (2015) included the only opecoelinine

that has been sequenced to date, *Buticulotrema thermichthysi* Bray, Waeschenbach, Dyal, Littlewood, & Morand, 2014 in their phylogenetic analysis, and found that it nested in one of the plagioporine clades that also contained opecoeline species. Andres et al. (2014) and Bray et al. (2014) concluded that the small sample of opecoelid sequences available precluded their ability to amend subfamilial designations. Notably, the type genera of the Plagioporinae, Opecoelininae, Opecoelinae, and Stenakrinae have yet to be included in molecular phylogenies of adult opecoelids.

Morphologically distinguishing opecoelid genera has long been and continues to be difficult and confusing; many taxa of opecoelids morphologically grade into one another without any definitive distinguishing characters. Moreover, characters are applied inconsistently in the family such that a given character will be used in one instance to distinguish species and in another used to distinguish genera. Both unnatural lumping and subdivision of genera has likely occurred (Cribb, 2005). In the case of splitting, of the 87 genera of opecoelids known, 23 are monotypic (Cribb, 2005). Many of these monotypic genera could be considered of marginal value *sensu* Cribb (2005).

Although most opecoelid genera are present in either marine or estuarine fishes, a small subset of genera (16) representing over 75 species are specific to or also parasitize freshwater fish (Cribb, 2005). Invasion of freshwater hosts has occurred at least two times based on the most recent phylogenetic analyses, with separate invasions apparent for the plagioporines (represented by *Plagiocirrus loboides* Curran, Overstreet, & Tkach, 2007) and the opecoelines (represented by *Dimerosaccus oncorhynchi* [Eguchi, 1931] Shimazu, 1980) (Andres et al., 2014; Bray et al., 2014, 2016; Shedko et al., 2015). As this dissertation is focused on the taxonomy and systematics of plagioporines from

freshwater hosts, these taxa will now be briefly discussed, with separate reviews for Palearctic/Indo-Malyan and Neartic genera. Nearctic genera will be reviewed in greater depth as this dissertation is mostly on plagioporines from this ecozone.

Plagioporine Genera Occurring in Freshwater of the Palearctic/Indo-Malaya

There are 11 plagioporine genera with members occurring in freshwater of the Holarctic/Indo-Malaya (*sensu* Cribb, 2005). Collectively these genera display a range of intestine conformations. *Nicolla* Wisniewski, 1933 is the only of these genera with caeca that form a cyclocoel and its members parasitize a wide range of freshwater and marine fish families. Although the distribution of this genus is listed as cosmopolitan, its members parasitizing freshwater fishes are restricted to the Palearctic. *Vesicocoelium* Tang, Hsu, Huang, and Lu, 1975 also parasitizes both freshwater and marine fish of several families, but unlike *Nicolla*, has caeca that unite to form a common anus. *Vesicocoelium* is distributed in the Indian and Pacific oceans, with members parasitizing several families of freshwater and marine fish. The caeca of *Neopecoelina* Gupta, 1953 form an uroproct. Members of this genus are restricted to freshwater fishes (Siluriformes) of India. The remaining 8 freshwater plagioporine genera of the Palearctic/Indo-Malaya have blindly terminating caeca and are found only in freshwater fish. Six of these genera have limited distributions in the Palearctic; these include *Neoplagioporus* Shimazu, 1990 from several families of fish in Japan and Korea, *Urorchis* Ozaki, 1927 from several families of fish in China and Japan, *Eucreadium* Dayal, 1950 from several families of fish in India, *Neopodocotyle* Dayal, 1950 from several families of fish in India, *Pseudosphaerostomum* Koval and Shevchenko, 1970 from cyprinids in Europe (monotypic), and *Pseudurorchis* Yamaguti, 1971 from several families of fish in Israel.

The remaining two genera have distributions spanning the Palearctic and include *Sphaerostoma* Rudolphi, 1809 from several families of freshwater fish primarily in Europe but also occasionally in Asia and *Plagioporus* Stafford, 1904 also from several families of freshwater fish. Of the 11 genera of freshwater plagioporines distributed in the Palearctic/Indo-Malaya, only 2 of these genera, *Plagioporus* and *Pseudurorchis*, are distributed in the Nearctic (Cribb, 2005).

Plagioporine Genera Occurring in Freshwater of the Nearctic: *Plagioporus* Stafford, 1904

Seven freshwater plagioporine genera, all of which have blindly ending caece, have species occurring in freshwater habitats of the Nearctic, of which *Plagioporus*, the type genus of the Plagioporinae, is the most speciose with 13 species (Cribb, 2005; Tracey et al., 2009).

Plagioporus was erected to accommodate *Plagioporus serotinus* Stafford, 1904, which was collected from the intestine of *Moxostoma macrolepidotum* (Lesueur) obtained from a fish market in Montreal, Canada. In the Nearctic, there are 13 valid species in the genus *sensu* Cribb 2005 and Tracey et al. (2009), including 11 intestinal and 2 gall bladder species. Gall bladder species include *Plagioporus serratus* Miller, 1940 from *Hiodon tergisus* Lesueur from the St. Lawrence River, Montreal, Canada, and *Plagioporus sinitsini* Mueller, 1934 from the gall bladder of *Catostomus commersonii* (Lacepède) from Oneida Lake, New York. Intestinal species apart from *P. serotinus* include the following species from their type hosts and localities: *Plagioporus angusticollis* (Hausmann, 1896) from *Cottus gobio* Linnaeus from Europe (but subsequently reported by Haderlie [1953] from *Oncorhynchus mykiss* [Walbum] from

California), *Plagioporus cooperi* (Hunter & Bangham, 1932) Price, 1934 from various cyprinids from Lake Eerie, *Plagioporus hypentelli* Hendrix, 1973 from *Hypentelium nigricans* (Lesueur) from Marsh Creek, Pennsylvania, *Plagioporus macrouterinus* Haderlie, 1953 from *Ptychocheilus grandis* (Ayres) from Deer Creek, California, *Plagioporus shawi* (McIntosh, 1939) Margolis, 1970 from *Oncorhynchis kisutch* (Walbum) from the Alsea River, Oregon, *Plagioporus siliculus* Sinitsin, 1931 from *Oncorhynchus clarkii* (Richardson) from Oak Creek near Corvalis, Oregon, *Plagioporus lepomis* Dobrovolny, 1939 from *Lepomis megalotis* (Rafinesque) from the Huron River, Michigan, *Plagioporus chiliticorum* (Barger & Esch, 1999) Cribb, 2005 from *Notropis chiliticus* (Cope) from Basin Creek, North Carolina, *Plagioporus boleosomi* (Pearse, 1924) Peters, 1957 from *Etheostoma nigrum* Rafinesque from Lake Pepin, Wisconsin and *Plagioporus kolipinskii* Tracey, Choudhury, Cheng & Ghosh, 2009 from *Gasterosteus aculeatus* Linnaeus from Lobos Creek, California (Tracey et al., 2009). Collectively, species of *Plagioporus* in the Nearctic parasitize cyprinid, percid, catostomid, salmonid, hiodontid, centrarchid, and gasterosteid hosts.

The life cycles of 6 of the 13 Nearctic species of *Plagioporus* have been described. These include 4 species, *P. sinitsini*, *P. hypentelli*, *P. lepomis*, and *P. siliculus*, with cerithioidean first intermediate hosts and two additional species, *P. shawi* and *P. angusticolle*, that respectively use hydrobiid and neritid first intermediate hosts (Dobrovolny, 1939a, 1939b; Hendrix, 1978; Sinitisin, 1931; Mathias 1936, 1937; Schell 1974, 1975).

The life cycle of *P. sinitsini* was reported by Dobrovolny (1939a). Cercariae of *P. sinitsini* develop in sporocysts in the gonoduct of *Elimia livescens* (Menke) and

subsequently encyst and develop into metacercariae *in situ*. These sporocysts containing infective metacercariae are then shed by the snail and consumed either by cyprinid or catostomid definitive hosts. Metacercariae of *P. sinitsini* were fed to a variety of fish, and those in which this species reached maturity were *Nocomis biguttatus* (Kirtland), *Luxulis cornutus* (Mitchill) and *Poecilia reticulata* Peters. Within 25 hours after feeding metacercariae, some of the worms had migrated to the gall bladder and worms became gravid between 15 and 30 days post-infection (Dobrovolny, 1939a). The life cycle of *P. hypentelli* was reported by Hendrix (1978). Cotylomicrocercous cercariae are shed after 106 days following infection of the first intermediate host, *Leptoxis carinata* (Bruquiere), with miracidia. These cercariae penetrate a variety of macroinvertebrates, including alderflies, dipterans, caddisflies, damselflies, mayflies and amphipods, but only encyst in *Sialis infumata* Newman under natural conditions and *Culex pipiens* Linnaeus under experimental conditions. Infected *C. pipiens* were fed to *Xiphophorus helleri* Heckel, and although mature adults were not obtained, two of the four worms obtained after 5 days (maximum worm maintenance in *X. helleri*) had spermatozoa in the seminal vesicle and were reported to be identical to immature worms collected naturally from the type host (Hendrix, 1978). Similar to *P. hypentelli*, cotlyomicrocercous cercariae of *P. lepomis*, which shed from *E. livescens*, penetrate a wide range of arthropod hosts but only developed to the infective stage in *Hydroporus sp*. and *Hyalella azteca* (Saussure), the latter of which being the most commonly found naturally infected host. Infected amphipods were fed out to a variety of local fishes including cyprinids, percids, and catostomids; sexually mature worms were only obtained from centrarchids (Dobrovolny, 1939b). The cercariae of *P. siliculus*, which shed from *Juga plicifera* (Lea), seem to

exhibit more specificity with respect to the second intermediate host; although cercariae were exposed to young fish, snails, and insect larvae, they only penetrated and encysted in *Potamobius* sp., with some individuals achieving progenesis. Experimental infections with definitive hosts were not carried out (Sinitisin, 1931).

P. shawi is the only species of *Plagioporus* from the Nearctic with a hydrobiid first intermediate host. Within 95-130 days of miracidial penetraton of *Fluminicola gustafsoni* Hershler & Liu, cotylomicrocerous cercariae are shed. Cercariae penetrate and encyst in amphipods, chironomids, caddisflies, stoneflies and mayflies, with the latter two being the only naturally infected hosts found. Experimental infections of the definitive hosts (6 hatchery bred *O. mykiss* 5-6 inches in length) using infected amphipods were unsuccessful in producing sexually mature worms. The oldest worms recovered from these infections were 26 days old, at which point the worms were 1.0-1.5 mm in length (smallest length of mature worms from natural infections is 2.3 mm) (Schell, 1975).

The life cycle of *P. angusticollis* has only been reported from Europe. Cotylomicrocercous cercariae are shed by *Theodoxus fluviatilis* (Linnaeus) and encyst in *Gammarus pulex* (Linnaeus) and *Asellus aquaticus* (Linnaeus). Infected *G. pulex* were fed to *Anguilla anguilla* (Linnaeus) and *C. gobio*; both hosts yielded sexually mature adults (Mathias 1936; 1937).

Other Plagioporine Genera in Freshwaters of the Nearctic

Plagiocirrus Van Cleave & Mueller, 1932 was erected to accommodate *P. primus* Van Cleave & Mueller, 1932 collected from the intestine of *Notemigonus crysoleucas* (Mitchill) from Oneida Lake, New York (Van Cleave & Mueller, 1932). *Plagiocirrus* is

distinguished from closely allied genera (*sensu* Cribb 2005) primarily by the vitelline follicles being restricted to a short field in the anterior half of the hindbody and in having the uterus extend to the posterior end. Two additional species of *Plagiocirrus* have been described from the Nearctic: *P. testeus* Fritts, 1959 from the intestine of *Catostomus macrocheilus* Girard, 1856 from the Clearwater River, Idaho and *P. loboides* Curran, Overstreet, & Tkach 2007 from intestine of *Fundulus notti* (Agassiz) (also reported from other fundulids and *Notemigonus crysoleucas* [Mitchill]) from the Pascagoula River, Mississippi (Fritts 1959; Curran, Overstreet, & Tkach, 2007). The remaining plagioporine genera from freshwater hosts of the Nearctic are either monotypic (*Multvitellina* Schell, 1974, *Nezpercella* Schell, 1974 and *Pseudopodocotyle* Caballero Rodriguez, 1970) or only have a single member parasitizing Nearctic freshwater fishes (*Allopodocotyle* Pritchard, 1966 and *Pseudurorchis* Yamaguti, 1971) (Cribb, 2005).

Schell (1974) described 2 new genera 3 new species of opecoelids from freshwater fishes of Idaho. The new genera erected were *Multivitellina* for *Multivitellina idahoensis* Schell, 1974 collected from the intestine of *Ptychocheilus oregonensis* (Richardson) and *Richardsonius balteatus* (Richardson) from Payette River and Payette Lake, Idaho, and *Nezpercella* for *Nezpercella lewisi* Schell, 1974 collected from the intestine of *P. oregonensis* from the Clearwater River, Idaho. The third species, *Pseudurorchis catostomi* Schell, 1974 was described from the intestine of *Catostomus macrocheilus* from the Clearwater River, Idaho. The type species of *Pseudurorchis* (*sensu* Cribb 2005) is *Pseudurorchis lacustris* (Paperna, 1974) described from the intestine of *Blennius vulgaris* Pollini and *Garra rufa* (Heckel) from Israel. *Multivitellina*, *Nezpercella* and *Pseudurorchis* can be distinguished from *Plagioporus* in having the

uterus extending to the posterior end of the body, from *Plagiocirrus* by the greater extent of the vitelline follicles (*sensu* Cribb 2005), and from each other by a combination of the extent of the vitelline follicles, ratio of suckers, position of the testis, and the position and shape of the ovary (Schell 1974).

Allopodoctyle virens (Sinitsin, 1931) Pritchard, 1966 was described from the Northwest and was found in the intestine of *Cottus* sp. from the Siouslow River near Mapleton, Oregon. *Allopodocotyle* Pritchard, 1966 can be distinguished from *Plagioporus* by having an excretory bladder extending to the level of the ovary and in its parasitism of marine fishes (*sensu* Cribb 2005). Interestingly, the definitive host of *A. virens* is a freshwater cottid (Sinitsin, 1931).

Of these 5 genera and the species they contain, the life cycle has only been reported for *N. lewisi* and *A. virens* (Schell, 1976; Sinitsin, 1931). In the case of *N. lewisi*, cotylomicrocercous cercariae are shed by *F. gustafsoni* within 105 days of exposure to miracidia. Although cercariae were exposed repeatedly to 18 different aquatic invertebrate taxa, penetration and encystment only occurred in *O. mykiss*, *Cottus rhotheus* (Smith) and various local species of cyprinids. Infected cyprinids were fed to 2 adult *P. oregonensis*. After 8 weeks, the largest *N. lewisi* recovered was 1.8 mm in length with weakly developed vitelline follicles and a developing uterus without eggs; the size range for sexually mature, naturally obtained adults was noted to be 2.0-2.6 mm (Schell, 1976). The cercariae of *A. virens* is also shed from a hydrobiid, *Fluminicola virens* (Lea). The cercariae encyst either in *F. virens* (presumably the soft tissue) or preferentially in the adolescariae of other species of digeneans. It is to be noted that the type locality for the

adult and first intermediate host of *A. virens* are in different drainages, and that no experimental infections using the definitive host were undertaken (Sinitsin, 1931).

The only other opecoelid genus reported from the Nearctic from freshwater is *Pseudopodocotyle*. The type and only species, *P. bravoae* Caballero Rodriguez, 1970, is only known as a metacercariae in Mexican freshwater crustaceans (Cribb, 2005). Cribb (2005) notes that although it is unfortunate to establish new genera from immature specimens, it is clear that this species does not agree well with any known opecoelid genus given the combination of its short caeca that do not pass posteriorly beyond the anteriorly located testis, the three to four-lobed ovary, the anterior location of the genital pore and the distribution of vitelline follicles.

Remarks on *Plagioporus* and Other Freshwater Plagioporines

Stafford (1904) provides a very brief description of *P. serotinus* (the type species of *Plagioporus*) and does not discuss the erection of *Plagioporus*, though its etymology indicates an obliquely positioned genital pore. Miller (1941) subsequently redescribes *P. serotinus* from the Ottawa River near its confluence with the Saint Lawrence River, the probable type locality, from both *Moxostoma* (reported as 'red horse sucker, M. aureolum'- most likely *M. macrolepidotum*) and also *Catostomus commersonii* (Lacepède). Subsequent to its erection, *Plagioporus* was continually confused with marine opecoelid genera; over 100 species have been assigned to this genus, with many having been subsequently placed in one of at least 9 morphologically similar marine genera (Cribb 2005; Gibson & Bray, 1982). The first major reorganization of the *Plagioporus*-complex was provided by Gibson (1976), who differentiated *Plagioporus* from two marine genera, *Podocotyle* Dujardin, 1845 and a newly erected genus,

Neolebouria Gibson, 1976, based on distribution of the vitellarium in the forebody and the nature of the ovary. Shortly thereafter, Bray (1979) argued that Yamaguti (1934) had introduced an erroneous conception of *Caudotestis* Issaitschikov, 1928 and considered this marine genus, which had been considered by many authors as a subgenus or synonym of *Plagioporus*, monotypic and a member of the Stenakrinae. The most recent and widely accepted revision of *Plagioporus* was by Gibson & Bray (1982), who restricted this genus to freshwater forms with a short excretory vesicle, reaching anteriorly at most to the level of the posterior testis, thereby distinguishing it from marine forms with which it had been historically confused that have excretory vesicles generally reaching the level of the ovary. As a result, numerous species originally described in *Plagioporus* were reallocated to the following genera: *Allopodocotyle*, *Gaevskajatrema* Gibson & Bray, 1982, *Neolebouria*, *Podocotyle* and *Macvicaria* Gibson & Bray, 1982, with *Macvicaria* receiving the most species (over 15) (Cribb, 2005). *Macvicaria* was erected for marine species of *Plagioporus* (*sensu lato*) possessing a ventrolateral genital pore and an excretory vesicle reaching anteriorly at least to the level of the anterior testis (Gibson & Bray, 1982).

While the revision of Gibson & Bray (1982) added much needed clarity to *Plagioporus* complex, the distinction of *Plagioporus* from marine forms continues to be problematic. Following the taxonomic changes of Gibson & Bray (1982), Gibson (1996) commented that the short excretory vesicle of 2 of the 3 North American species of *Plagioporus* from freshwater fish at that time maintained in the genus *Allopodocotyle* may require separation from the marine representatives of this genus. Cribb (2005) transferred these two species, *P. lepomis* and *P. boleosomi*, along with the subsequently

described species *P. chiliticorum*, which was originally placed in *Allopodocotyle* by Barger & Esch (1999), to *Plagioporus*, noting that 'the possession of a short excretory bladder and parasitism of freshwater fishes probably indicates a relationship to *Plagioporus*.' The third species of *Allopodocotyle* from North American freshwater fish addressed by Gibson (1996) was *A. virens*, which was originally described in the genus *Plagioporus*. Gibson (1996) distinguished *A. virens* from *P. lepomis* and *P. boleosomi*, its freshwater congeners at the time, by its large size and a long excretory bladder that reaches the level of the ovary. Cribb (2005) uses this later detail as justification for retaining *A. virens* in *Allopodocotyle*, despite the restriction of *Allopodocotyle* to marine fish by the same author. Interestingly, Cribb (2005) does not address *P. shawi*, which is of similar body size. Despite its long excretory vesicle that extends anteriorly well beyond the testis, *P. shawi* is retained in *Plagioporus*. Hence, the generic criterion for both *Allopodocotyle* and *Plagioporus* have been inconsistently applied. In addition to *P. shawi*, *P. siliculus* also has an excretory vesicle reaching the level of the anterior testis, which violates the diagnosis of *Plagioporus* proposed by Gibson & Bray (1982).

In addition to not being well differentiated from marine forms, *Plagioporus* is also very similar in morphology to other freshwater plagioporine genera possessing blindly ending caeca, often only being distinguished from another given genus by one to two characters. Only two characters, for example, can be used to differentiate members of *Plagiocirrus* from *Plagioporus*, including a reduced vitelline field and a posteriorly extending uterus (Cribb, 2005). However, both of these characters are represented in members of *Plagioporus*. The vitelline field of *Plagiocirrus loboides* is restricted to two lateral bands occurring between the anterior margin of the ventral sucker and the middle

of the posterior testis (Curran et al., 2007). *Plagioporus chiliticorum* has a similarly reduced vitelline field, with the anterior and the posterior extent occurring at the anterior margin of the ventral sucker and slightly beyond the posterior testis, respectively (Barger & Esch, 1999). Although most species of *Plagioporus* have a pretesticular uterus, *P. macrouterinus* and *P. chiliticorum* have a uterus that respectively extends as far posteriorly as the middle of the posterior testis and to the anterior margin of the posterior testis (Barger & Esch, 1999; Haderlie, 1953). *Plagioporus* is also very similar in morphology to *Neoplagioporus*, *Urorchis* and *Sphaerostoma*. *Neoplagioporus* only differs morphologically from *Plagioporus* in possession of a contiguously bipartite internal seminal vesicle (Cribb, 2005); while the internal seminal vesicle of *P. chiliticorum* is bipartite, it is not contiguously bipartite as the chambers of the seminal vesicle are separated by a distinct duct (Barger & Esch, 1999). *Urorchis* also differs from *Plagioporus* in possession of a contiguously bipartite internal seminal vesicle along with a uterus extending posteriorly to the end of the body. Similarly, *Sphaerostoma*, is also not clearly differentiated from *Plagioporus* (Cribb, 2005). While *Sphaerostoma* has a uterus passing between the testes, both *P. chilitcorum* and *P. macrouterinus* can have uterine loops at the junction of the testes (Barger & Esch, 1999; Haderlie, 1953). *Sphaerostoma* also has an ovary that is either between the testes or lateral to the anterior testis (Cribb, 2005). Though most species of *Plagioporus* have an ovary that is oblique to or tandem with the anterior testis, the ovary of *P. cooperi* is nearly parallel to the anterior testis (Hunter & Bangham, 1932). The monotypic genera *Multivitellina* and *Nezpercella* can only be differentiated from *Plagioporus* by possession of a uterus extending to the posterior end of the body. *Pseudurorchis* has a similar uterus, though its posterior extent

is only slightly beyond that of *P. macrouterinus* relative to the posterior testis (Cribb, 2005; Haderlie, 1953). In addition, like *Urorchis* and *Neoplagioporus*, *Pseudurorchis* has a contiguously bipartite internal seminal vesicle (Cribb, 2005).

Pseudurorchis and *Plagioporus* are unique among freshwater plagioporines in having members distributed in both the Palearctic and Nearctic regions. *Pseudurorchis* is very likely to be an unnatural grouping given its disjunct distribution; its only members include 2 species collectively parasitizing cyprinid, cyprinodontid, and blenniid fishes of Israel and a single species, *P. catosomi*, from a catostomid in Idaho, U.S.A. It is very likely that *P. catostomi* will require transfer to another genus. Unlike *Pseudurorchis*, *Plagioporus* is distributed throughout both the Palearctic and Nearctic. Notably, it is by far the most speciose freshwater plagioporine genus, with 31 species in the Holarctic region (the next most speciose generea are *Nicolla* [n=14] and *Eucreadium* [n=8]) (Cribb, 2005).

Given that *Plagioporus* was erected for a species parasitzing a freshwater fish in the Nearctic, it is possible that it represents a Nearctic genus and that members distributed in the Palearctic belong in genera specific to this ecozone like *Sphaerostoma* and or *Neoplagioporus*, from which *Plagioporus* is not clearly differentiated (Cribb, 2005). Following the revisions of Gibson & Bray (1982), *Plagioporus* from the Palearctic total 18 species, including *P. acerinae* (Pigulewskii, 1931), *P. allovaris* Zhang, 1992, *P. angusticolle*, *P. bilaris* Paperna, 1964, *P. gibsoni* Bilqees, Shaikh, & Khan, 2010, *P. glomeratus* Roytman, 1963, *P. gonii* Bilqees & Khan 1988, *P. honshuensis* Moravec & Nagasawa, 1998, *P. imanensis* Belouss, 1958, *P. nemachili* Paperna, 1964, *P. occidentalis* Szidat, 1944, *P. protei* Prudhoe, 1945, *P. schizothoraci* Zhang, 1992, *P.*

sichuanensis Wang, 1985, *P. sindhensis* Shaikh & Bilqees, 2008, *P. skrjabini* Koval, 1951, *P. stefanski* Slusarski, 1958, and *P. triangulogenitalis* Belouss, 1958.

Although *Plagioporus* is more speciose in the Palearctic (18 compared with 13 species), there are indications that the diversity of *Plagioporus* in the Nearctic is greatly underestimated (Cribb, 2005, Tracey et al., 2009). An additional 4 new species of *Plagioporus* were described by Aliff (1973) from Kentucky, including 2 intestinal species, '*Podocotyle etheostomae*' from *Etheostoma blennioides* Rafinesque (mistakenly placed in a strictly marine genus by Aliff [1973]) and 'Plagioporus elongatus' from *Pimephales notatus*, a gall bladder form, 'Plagioporus notropidus' from *Lythrurus ardens* (Cope) and *Semotilus atromaculatus* (Mitchill), and a species that occurs only as an adult in its first intermediate pleurocerid snail host, 'Plagioporus neotenicus.' However, Aliff's (1973) thesis was never formally published and hence according to the ICZN, these species are *nomina nuda*. An additional *nomen nudum* species, 'Plagioporus tennesseensis', from the intestine of *Campostoma anomalum* (Rafinesque) in Tennessee was named and deposited in the USNPC by Leon Duobinis-Gray (Tracey et al., 2009). Tracey et al. (2009) note that 'P. tennesseensis' has a more tubular excretory vesicle than those of its congeners in cyprinids. Several species of *Plagioporus* reported by Haderlie (1953) from freshwater fishes of California also seem to represent new species. *Plagioporus serotinus* of Haderlie (1953), for example, from the gut of the only native extant centrarchid west of the Rocky Mountains, *Archoplites interruptus* (Girard), is likely a misidentification and represents an undescribed species; the shape of the cirrus sac and size of the eggs of this form are different from those of *P. serotinus* and the host is not a catostomid (Manter, 1954). In addition, Haderlie (1953) considered *Plagioporus*

sp. from the intestine of *O. mykiss* to represent a new species, but refrained from naming it as he only had 5 specimens available for study. An additional form reported by Haderlie (1953) from the same host in a different drainage, *Plagioporus angusticollis*, represents a misidentification and likely a new species distinct from *Plagioporus* sp. *Plagioporus angusticollis* was described by Hausman (1896) in Europe and the life cycle was reported by Mathias (1936, 1937) also from Europe. Its first intermediate host, *Theodoxus fluviatilus* (Linnaeus) (previously *Nerita fluviatilis*) is in the family Neritidae Rafinesque. The distribution of *Theodoxus* Montfort is restricted to Europe and Eastern Asia. It would be plausible that Haderlie (1953) found *P. angusticollis* in California if members of Neritidae were found on the Pacific coast of the US, but this is not the case; no nertids are found on the West coast; they are restricted to the Atlantic in North America (Turgeon et al., 1998). Furthermore, the specimens reported by Haderlie (1953) are morphologically inconsistent with *P. angusticollis*; Manter (1954) notes that the anterior extent of the vitellarium and short cirrus sac of the specimens from California are not shared by *P. angusticollis*. An additional form very similar to 'Plagioporus neotenicus' of Aliff (1973) was reported by Barger & Esch (2000) from Basin Creek, North Carolina. These authors found adult *Plagioporus* sp. in daughter sporocysts in *Pleurocera proxima* (Say) (reported as *Pleurocera symmetrica*) and in the gall bladder of *Clinostomus funduloides* Girard from Basin Creek, and identified the specimens as *P. sinitsini*. According to these authors, adults of *P. sinitsini* from snails and fish were morphometrically indistinguishable (Barger & Esch, 2000). The photomicrographs of the form maturing in snails taken by Barger & Esch (2000), however, are inconsistent with the morphology of *P. sinitsini*, which has a distinctly spindle-shaped body. Most recently,

McAllister et al. (2014a) reported *Plagioporus* sp. from *Noturus lachneri* Taylor, from the Middle Branch of Gulpha Creek in the upper Ouachita River drainage, Arkansas. Subsequently, McAllister et al. (2015) reported *Plagioporus* sp. from the intestine of an additional madtom species, *Noturus exilis* Nelson, from Flint Creek, Arkansas, and noted that they could not morphologically distinguish their specimens from *Plagioporus* sp. from the intestine of *Cottus carolinae* (Gill) reported by McAllister et al. (2014b) also collected from Flint Creek, Arkansas. Upon their description, both the Flint and Gulpha Creek forms would be the first species of *Plagioporus* known to parasitize ictalurids. Moreover, the Flint Creek form is the first record of *Plagioporus* from *Cottus* in the eastern Nearctic.

Apart from undescribed species of *Plagioporus* from fish and snail hosts, undocumented diversity of *Plagioporus* may exist in amphibians. *Plagioporus gyrinophili* Catalano & Etges, 1981 was described from the intestine of the salamanders *Gyrinophilus porphyriticus duryi* (Weller) and *Pseudotriton ruber* (Latreille) from Ohio, and was subsequently reported from the intestine of *Eurycea spelaea* Stejneger from Missouri (Catalano & Etges, 1981; McAllister et al., 2006). This species has been entirely neglected in taxonomic works on *Plagioporus* since its description, possibly due to its atypical host. However, species of *Neoplagioporus*, *Dimerosaccus* Shimazu, 1980 and *Plagioporus* have been reported from salamanders in Korea, Japan and Slovenia, respectively (Cribb 2005; Prudhoe, 1945; Shedko et al., 2015).

Molecular Studies of Freshwater Plagioporines

Of the over 75 species of freshwater plagioporines distributed across 16 genera, only a single species in the genus *Plagiocirrus*, *P. loboides*, has been included in

molecular phylogenies of the opecoelids (Andres et al., 2014; Bray et al., 2014, 2016; Olsen et al., 2003; Shedko et al., 2015). The most recent molecular phylogenies of the opecoelids have resolved *P. loboides* as the highly supported sister to a clade containing deep water marine opecoelids, including the opecoelinine *Buticulotrema thermichthysi* Bray, Waeschenbach, Dyal, Littlewood, & Morand, 2014 and the plagioporine genera *Gaevskajatrema*, *Neolebouria* and *Podocotyloides* Yamaguti, 1934. The clade containing all of these species was in turn sister to one composed of opecoelines (represented by *Opecoeloides* Odhner, 1928 and *Dimerosaccus* [Eguchi, 1931] Shimazu, 1980) (Andres et al., 2014; Bray et al., 2014; Shedko et al., 2015). Interestingly, Bray et al. (2016) hypothesized that members of *Plagioporus* will be closely related to *Plagiocirrus* based on their common parasitism of freshwater hosts, but in their cladogram labeled the clade composed of *Plagiocirrus*, the deep sea plagioporines and the only sequenced opecoelinine as 'Plagioporinae,' despite *Plagioporus* being the type genus of the Plagioporinae.

Hypotheses

Given their similar morphology and common parasitism of freshwater fishes, we hypothesize that the freshwater plagioporines, including *Plagioporus*, form a monophyletic group. This hypothesis is primarily tested in Chapter 2 in which we conduct a BI analysis of the partial 28S rDNA gene including the following opecoelids: 1) 5 species of *Plagioporus* previously described from freshwater fishes of the Nearctic; 2) a new species of *Plagioporus* from the intestine of *O. mykiss* from California; 3) 3 species of *Neoplagioporus* from Japan, including the type species, *Neoplagioporus zacconis* (Yamaguti, 1934) Shimazu, 1990; 4) 2 species of *Urorchis* from Japan,

including the type species, *Urorchis goro* Ozaki, 1927; 5) and 26 other opecoelids from GenBank, including *P. loboides*. The monophyly of the freshwater plagioporines is further assessed in Chapters 3-6, in each of which new species of *Plagioporus* (10 new species altogether for these chapters) are described using morphological methods and added to the BI analysis from Chapter 2.

We further hypothesize that *Plagioporus* has radiated in the intestine across a range of fish families with freshwater/anadromous members in the Nearctic. In Chapter 2 this hypothesis is tested with a BI analysis of the 28S rDNA gene that includes sequences of 6 species of *Plagioporus* collectively from fundulid, salmonid, gasterosteid, percid, and cyprinid hosts. In Chapter 3, forms from ictalurid and cottid hosts representing new species are added to the BI analysis, including one from *Cottus carolinae* and *Noturus exilis* from Flint Creek, Arkansas, and another from *Noturus lachneri* from the upper Ouachita River drainage, Arkansas. In Chapter 6, forms from catostomids also representing new species are added to the BI anaylsis. Morphological methods are used to describe new species of *Plagioporus* and to assess their morphology relative to congeners parasitizing other fish families with freshwater/anadromous members in the Nearctic.

Intestinal *Plagioporus* of the Nearctic are also hypothesized to have radiated within a range of fish families with freshwater/anadromous members, particularly for the percids, cyprinids, and catostomids. In Chapters 4, 5, and 6 potential radiations of intestinal *Plagioporus* are examined for the percids, cyprinids, and catostomids, respectively, with 3, 2, and 3 new species respectively described for each freshwater fish family. New species of intestinal *Plagioporus* from percids are respectively from *Etheostoma blennioides newmanni* Miller from Big Creek, Arkansas, *Etheostoma*

squamosum Dislter from Flint Creek, Arkansas, and *Percina nigrofasciata* Agassiz from Alexander Spring, Florida. New species of intestinal *Plagioporus* from cyprinids include one from *Rhinichthys* spp. from Cosby Creek in the Great Smokey Mountains, Tennessee and another from *Clinostomus funduloides* from Crooked Creek, Virginia. New species from catostomids are all from *H. nigricans* respectively from Cosby and Abrams Creeks, Great Smokey Mountains, Tennessee, and Crooked Creek, Arkansas. Previously described species from each of these three families are redescribed with newly collected material when available, including *P. boleosomi* from *E. nigrum, Percina caprodes* (Rafinesque), and *Percina maculata* (Girard) from West Twin Creek, Wisconsin and *P. chiliticorum* from *N. chiliticus* from Basin Creek, North Carolina. In Chapter 2, radiation of *Plagioporus* in the intestine of salmonids is also assessed. In each chapter, we use morphological and molecular methods to assess whether a given radiation is monophyletic, or in other words whether each radiation represents a single host switching event into a given fish family or multiple, independent events.

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CHAPTER II – Amendment of *Plagioporus* Stafford, 1904 (Digenea: Opecoelidae), with a description of a new species from California and a phylogeny of freshwater plagioporines of the Holarctic

Abstract

Plagioporus hageli n. sp. is described from the intestine of *Oncorhynchus mykiss* (Walbaum) collected from the Yuba River, California, USA. Of the accepted, nominal species of *Plagioporus* Stafford, 1904 from the Nearctic, the new species is morphologically similar to three intestinal species from the western USA parasitising secondary freshwater fishes, including *Plagioporus shawi* (McIntosh, 1939), *Plagioporus kolipinskii* Tracey, Choudhury, Cheng & Ghosh, 2009 and *Plagioporus siliculus* Sinitsin, 1931, and is also similar to *Plagioporus serotinus* Stafford, 1904 from catostomids from eastern Canada. *Plagioporus hageli* n. sp. is distinguished from the former three species in lacking a dorsal vitelline field and from the latter species in having a consistent interruption in the distribution of the vitellarium at the level of the ventral sucker. The new species is also morphologically similar to an unnamed species of *Plagioporus* and a species misidentified as '*Plagioporus angusticolle*' that were collected from California, but it is easily distinguished from both in its shorter body length. To estimate the placement of the new species within *Plagioporus* and within the Opecoelidae Ozaki, 1925, I conducted a Bayesian inference (BI) analysis of partial 28S rDNA sequence data that included sequences from *Plagioporus hageli* n. sp., five other species of *Plagioporus*, three species of *Neoplagioporus* Shimazu, 1990 (including the type species, *Neoplagioporus zacconis* [Yamaguti, 1934]), two species of *Urorchis* Ozaki, 1927 (including the type species, *Urorchis goro* Ozaki, 1927) and sequences of 42 opecoelid

species obtained from GenBank.My phylogenetic estimation revealed 1) plagioporines parasitising freshwater hosts form a monophyletic group; 2) *Plagiocirrus loboides* Curran, Overstreet & Tkach, 2007 nestled within the rest of the members of *Plagioporus*; 3) the new species was closer to *Plagiocirrus loboides* than to *Plagioporus shawi*, the other salmonid parasite included in my analysis; 4) *P. shawi* was the poorly supported sister to its congeners; 5) *Neoplagioporus elongatus* (Goto & Ozaki, 1930) Shimazu, 1990 was closer to the two species of *Urorchis* than to the other two species of *Neoplagioporus*; and 6) the paraphyly of the Plagioporinae Manter, 1947 was reinforced. Based on 28S rDNA sequence data and my BI analysis, I propose *Plagioporus loboides* (Curran, Overstreet, & Tkach, 2007) comb. n., and amend *Plagioporus* accordingly. This analysis represents the first phylogenetic study of the opecoelids that estimates the interrelationships of the Plagioporinae that includes a member of *Plagioporus*.

Introduction

Plagioporus Stafford, 1904 was erected to accommodate *Plagioporus serotinus* Stafford, 1904 described from the intestine of *Moxostoma macrolepidotum* (Lesueur) obtained from a fish market in Montreal, Quebec, Canada. An additional 12 species of *Plagioporus* from the Nearctic were accepted by Tracey et al. (2009), with 10 intestinal species parasitising cyprinid, catostomid, percid, salmonid, gasterosteid, anguillid and centrarchid hosts, and two gall bladder species from cyprinid, catostomid and hiodontid hosts. Only two species of *Plagioporus* recognized by Tracey et al. (2009) from the Nearctic parasitize salmonids and both are distributed in the Pacific Northwest, namely *Plagioporus siliculus* Sinitsin, 1931 and *Plagioporus shawi* (McIntosh, 1939) Margolis, 1970. Previous molecular hypothesis of the Opecoelidae based on 28S rDNA sequence

data (e.g., Andres et al., 2014a; Bray et al., 2014, 2016; Shedko et al., 2015) have noted the paraphyly of the Plagioporinae Manter, 1947; however, none of those studies included a member of *Plagioporus*, the type genus of the subfamily. Shedko et al. (2015) suggested that the Plagioporinae should be restricted to a clade containing plagioporines from shallow-water marine perciform hosts (see Andres et al., 2014a). However, Bray et al. (2016) demonstrated that the two representatives of Opistholebetidae Fukui, 1929 were resolved in this same clade and emended the status of that family to the level of a subfamily within the Opecoelidae. *Plagiocirrus loboides* Curran, Overstreet, & Tkach, 2007 was the only freshwater 'plagioporine' representative included in all previous studies and was consistently resolved within a larger clade including deep-sea 'plagioporines' and opecoelines. Bray et al. (2016) suggested that species of *Plagioporus* likely would be resolved closer to *Plagiocirrus* because both represent freshwater genera whereas Shedko et al. (2015) considered this clade Opecoelidae incertae sedis. Therefore, the inclusion of *Plagioporus* in a phylogenetic context should help clarify the complex subfamilial interrelationships.

During a survey of digenean parasites of Californian freshwater fishes in May 2011, a species of *Plagioporus* was sampled from *Oncorhynchus mykiss* (Walbaum) from the south fork of Yuba River, California, USA, that differs markedly from known congeners in salmonids and other hosts. I describe the new species using morphological and molecular methods and use complete internal transcribed space region (ITS) -2 and partial 28S ribosomal DNA (rDNA) sequence data to examine the interrelationships of freshwater plagioporines from the Holarctic. I conduct a Bayesian inference (BI) analysis of partial 28S rDNA sequences obtained from newly collected material of six species of

Plagioporus from the Nearctic, including *P. shawi*; two species of *Urorchis* Ozaki, 1927 from Japan; and three species of *Neoplagioporus* Shimazu, 1990 from Japan as well as available opecoelid sequences from GenBank.

Material and Methods

On 25 May 2011, specimens considered herein to represent a new species of *Plagioporus* were collected from the south fork of the Yuba River, outside the town of Washington, California, USA, from the intestine of *O. mykiss* caught via fly fishing. Collection data for previously described species of *Plagioporus*, *Urorchis* and *Neoplagioporus* are displayed in Table 1. Specimens of opecoelids were excised from the intestine or the gall bladder of fish hosts with the aid of a fine paintbrush and transferred to and observed in a shallow dish containing 0.6% saline solution. Subsequently, the saline solution was removed from the dish to the point where worms were confined to shallow water, at which time, near boiling tap water was rapidly added to kill worms, minimizing curling post-fixation. Heat-killed worms were immediately transferred to 10% neutral phosphate buffered formalin for morphological examination and 95% ethanol for molecular analysis. Worms were stained in Mayer's haematoxylin or acetocarmine, dehydrated in a graded ethanol series, cleared in methyl salicylate and mounted permanently in Canada balsam or Damar gum. Specimens were deposited in the United States National Parasite Collection at the Smithsonian National Museum of Natural History (NMNH), Washington, D.C. (Table 1). Specimens were examined using bright-field and differential interference contrast optics on an Olympus BX 51 microscope and illustrated using an attached drawing tube. Measurements are given in micrometers (µm) and are expressed as those of the holotype followed by the range of

those of other specimens in parentheses. Ratios are expressed as ranges. The length and width of vitelline follicles are expressed as averages of 10 random follicles distributed throughout the body.

Genomic DNA was isolated for each species (see Table 1 for number of replicates) using Qiagen DNAeasy Tissue Kit (Qiagen, Inc., Valencia, California, USA) following the instructions provided. DNA fragments *c.* 2,550 base pairs (bp) long, comprising the 3′ end of the 18S nuclear rRNA gene, ITS region (including ITS1 + 5.8S + ITS2), and the 5′ end of the 28S rRNA gene (including variable domains D1–D3), were amplified from the extracted DNA by polymerase chain reaction (PCR) on a PTC-200 Peltier Thermal Cycler using forward primers ITSF (5′-CGC CCG TCG CTA CTA CCG ATT G-3′) or S20T2 (5'-GGT AAG TGC AAG TCA TAA GC-3') and reverse primer 1500R (5′-GCT ATC CTG AGG GAA ACT TCG-3′). These PCR primers and multiple internal primers were used in sequencing reactions. The internal forward primers were DIGL2 (5′-AAG CAT ATC ACT AAG CGG-3′), 300F (5′-CAA GTA CCG TGA GGG AAA GTT G-3′) and 900F (5′-CCG TCT TGA AAC ACG GAC CAA G-3′) and the internal reverse primers were 300R (5′-CAA CTT TCC CTC ACG GTA CTT G-3′), DIGL2R (5′-CCG CTT AGT GAT ATG CTT-3′) and ECD2 (5′-CTT GGT CCG TGT TTC AAG ACG GG-3′) (for primers see Littlewood et al., 2000; Tkach et al., 1999, 2000, 2001, 2003; Tkach & Snyder, 2007). The resulting PCR products were excised from PCR gels using QIAquick Gel Extraction Kit (Qiagen, Inc., Valencia, California, USA) following the manufacturer's instructions, cycle-sequenced using ABI BigDye™ chemistry (Applied Biosystems, Inc., Carlsbad, California, USA), ethanol-precipitated and processed on an ABI 3130 Genetic Analyzer™. Contiguous sequences from the

species were assembled and edited using Sequencher^{TM} (GeneCodes Corp., Ann Arbor, Michigan, USA, Version 4.10.1) and representative sequences were submitted to GenBank (Table 1). The boundaries between the 5.8S gene, ITS2 and 28S gene fragment were located using the ITS2 Ribosomal Database (Keller et al., 2009) and the boundaries between the 18S gene, ITS1 and 5.8S gene was estimated using the annotations of Andres et al. (2014b). Pairwise sequence comparisons of the ITS2 and 28S rDNA sequence data of *Plagioporus, Urorchis* and *Neoplagioporus* was calculated with MEGA v6 using the ‘‘compute pairwise differencesfunction,’’ with gaps treated using the ‘‘pairwise deletion’’ function (Table 3). For phylogenetic analysis, sequences of related species were obtained from GenBank (Table 2). The sequences were aligned using MAFFT version 6.611b (Katoh et al., 2005) with 1,000 cycles of iterative refinement and the *genafpair* algorithm. The resulting alignment utilized 53 opecoelids, an acanthocolpid, a lepcreadiid and an enenterid and used the brachycladiid *Zalophotrema hepaticum* Stunkard & Alvey, 1929 as the outgroup based its phylogenetic position relative to the Opecoelidae (Olson et al., 2003) and to be comparable with the phylogeny presented by Bray et al. (2016). Phylogenetic analysis of the data was performed using BI with MrBayes 3.1.2 software (Huelsenbeck & Ronquist, 2001). The best nucleotide substitution model was estimated with jModeltest-2 (Darriba et al., 2012) as general time reversible with estimates of invariant sites and gamma-distributed among site-rate variation (GTR + I + Γ). The following model parameters were used in MrBayes: nst = 6, rates = invgamma, ngen = 5,000,000 and samplefreq = 1,000. Burn-in value was 1,250 estimated by plotting the log-probabilities against generation and visualising plateau in

parameter values (sump burnin = 1,250), and nodal support was estimated by posterior probabilities (sumt) (Huelsenbeck et al., 2001) with all other settings left as default.

Description of *Plagioporus hageli* n. sp.

Opecoelidae Ozaki, 1925

Plagioporus Stafford, 1904

Plagioporus hageli n. sp.

Type- and only known-host: Oncorhynchus mykiss (Walbaum), rainbow trout (Teleostei: Salmonidae).

Type-locality: South fork of Yuba River, near Washington, California, USA (39°21'26.37" N, 120°44' 55.60" W).

Site: Intestine.

Prevalence: 2 of 3 hosts (67%).

Intensity: 21 in one host, 12 in other (mean 17).

Type-material: Holotype (USNM 1416782), Paratypes (USNM 1416783-1416785).

Representative DNA sequences: Partial 18S, entire ITS region, partial (D1–D3) 28S: GenBank accession no. KX553950, from 3 identical sequences.

Etymology: The specific epithet is named in memoriam of William Edward Hagel (uncle of TJF); a lifetime mechanical, medical and aeronautical engineer, who lived and died in the water and loved fly fishing, the means by which the type host was collected.

Description (Figs. 1–4)

[Measurements based on 10 gravid wholemounts.] Body white to yellow in life, lanceolate, with bluntly rounded ends, tapering anteriorly, widest at approximately 2/5 of body length (BL), 905 (851–1,198) long, 232 (233–313) wide. Oral sucker subterminal, 90 (83–115) long, 95 (92–129) wide. Ventral sucker not sunken, wider than long, 145 (132–183) long, 154 (151–205) wide; width representing 66 (61-69)% of body width. Forebody 333 (314–435) long, representing 37 (31-38)% of BL. Ratio of oral sucker to ventral sucker width 1:1.6 (1:1.5–1.8). Prepharynx 23 (21–42). Pharynx slightly separated from to overlapping posterior 1/4 of oral sucker, 49 (52–68) long, 62 (56–72) wide. Oesophagus 111 (103–167) long, representing 13 (11-17)% of BL. Caecal bifurcation anterior to ventral sucker at 258 (243–339), representing 29 (25–31)% of BL; postcaecal space 74 (70–145) long, representing 8 (7-13)% of BL.

Testes 2, tandem to oblique, contiguous, subequal; anterior testis 107 (90–124) long, 103 (93–127) wide, slightly overlapping sinistral caecum ventrally, with anterior margin at 530 (465–700) BL, representing 59 (55–63)% of BL; posterior testis 116 (106–143) long, 110 (98–154) wide, dorsal to anterior testis, ventrally overlapping dextral caecum, sometimes slightly overlapping sinistral caecum ventrally, with anterior margin at 606 (562–795) BL, representing 67 (65–73)% of BL. Postesticular space 170 (186–260), representing 20 (19–22)% of BL. Cirrus sac clavate, 207 (208–304) 1ong, representing 23 (22-28) % of BL, 48 (47-55) wide, overlapping anterior 1/2–3/4 of ventral sucker. Vasa efferentia uniting vas deferens at proximal end of cirrus sac. Internal seminal vesicle 101 (61–131) long, 38 (30–46) wide, occupying posterior 1/3–1/2 of sac

with a single 90º turn or with single coil, communicating with pars prostatica at 90º turn. Ejaculatory duct present, not clearly differentiated from pars prostatica. Genital pore ventrolateral, sinistral, 237 (219–273) from anterior margin of body, representing 26 (22–26)% of BL.

Ovary ovoid to kidney bean shaped, 76 (60–90) long, 62 (63–87) wide, dextrally oblique to anterior testis, slightly overlapping anterior testis in posterior 2/3 of length, overlapping dextral caecum ventrally, with anterior margin at 516 (468–664) BL, representing 57 (55-59)% of BL. Oviduct extending posterodorsally from anterior portion of ovary, joining with canalicular seminal receptacle; seminal receptacle median, dorsal to anterior testis, extending slightly posterior to level of ovary. Laurer's canal extending anteriorly from seminal receptacle, opening sinistral on dorsal surface at level of anterior margin of ovary. Mehlis' gland immediately anterior to testis. Ootype extending anteriorly from seminal receptacle, conspicuous at level of Mehlis' gland. Uterus extends posteriorly to anterior 1/4 of anterior testis to anterior margin of anterior testis, with anterior extent at level of the genital pore to approximately 1/4 of distance between genital pore and pharynx, containing 9 (1-41) eggs. Metraterm arising at level of anterior margin of ventral sucker, joining male complex dorsally at level of genital pore. Eggs 58 (56–69) long, 31 (31–41) wide. Vitellarium follicular, ventral to caeca, in 2 lateral bunches anterior to posterior margin of posterior testis and ventrally confluent in posttesticular space, with break in distribution on either side at level of ventral sucker, anterior extent at 231 (227–296) BL from anterior end, representing 26 (22–31)% of BL, posterior extent at 842 (810–1151), or 93 (90–96)% of BL. Follicles of vitellarium number 79 (82-140), with 20 (16-41) follicles posterior to posterior margin of posterior

testis, average length of 10 follicles 31 (30 ± 2, 27-35), average width 26 (29 ± 2, 25-34). Vitelline reservoir median, dorsal to anterior testis, ventral to seminal receptacle. Common vitelline duct joining ootype dorsally anterior to Mehlis' gland.

Excretory vesicle tubular, I-shaped, dorsal to vitellarium, ventral to and often overlapping caeca, extending anteriorly to level immediately posterior to posterior testis to overlapping posterior 1/3 of posterior testis, 216 (197–269) long, representing 21 (20–24)% of BL; pore terminal.

Remarks

Of the 12 valid North American species of *Plagioporus*, *Plagioporus hageli* n. sp. is morphologically most similar to *P. serotinus* from catostomids in eastern Canada and also to the only three congeners distributed west of the Rocky Mountains from secondary freshwater fishes, namely *P. shawi* and *P. siliculus* from salmonids and *P. kolipinskii* Tracey, Choudhury, Cheng & Ghosh, 2009 from a gasterosteid. The new species is similar to these four congeners in the body length-to-width ratio and in having the vitellarium confluent in the posttesticular space and extending anteriorly to approximately the level of the caecal bifurcation. *Plagioporus hageli* n. sp. is distinguished from these congeners as follows: from *P. shawi* in having an unlobed ovary, an excretory vesicle extending anteriorly only to the posterior testis (as opposed to one reaching the level of the ovary) and a cirrus sac not extending into the hindbody; from *P. kolipinskii* in having a ventral sucker representing 60–66% of the body width (as opposed to one spanning the width of the body) and a longer cirrus sac (representing 22–28% of the BL rather than 7–13% of the BL in *P. kolipinskii*); from *P. serotinus* in possessing an interruption in the vitellarium at the level of the ventral sucker, having the

anterior extent of the vitellarium halfway between the pharynx and ventral sucker (as opposed to one nearly reaching the level of the pharynx) and in the more posterior position of the caecal bifurcation (2/3 as opposed to 1/3 of the distance between the ventral sucker and the pharynx); and from *P. siliculus* in having a cirrus sac that overlaps the ventral sucker, an excretory vesicle extending anteriorly to the posterior testis as opposed to one reaching the anterior testis and a more posterior position of the caecal bifurcation (1/3 of the distance between the ventral sucker and pharynx in *P. siliculus*). Additionally, the vitellarium of *P. shawi*, *P. kolipinskii* and *P. siliculus* consists of both dorsal and ventral fields of follicles whereas that of the new species only has vitelline follicles distributed ventrally.

Haderlie (1953) examined the intestinal tract of individuals of *O. mykiss* collected from California and reported an undescribed species of *Plagioporus* and a species misidentified as '*P. angusticolle*'. Morphological comparison of *P. hageli* n. sp. to the species he reported (Table 4) suggests that the new species is also closely allied with them. Haderlie (1953) recovered 5 specimens of *Plagioporus* sp. that were collected from the Klamath River (*c.* 320 km from the type locality of *P. hageli* n. sp.) from a single host. He provided a brief description of those specimens but noted that the few specimens available did not constitute enough material to make an accurate determination of a new species. Voucher specimens of *Plagioporus* sp. could not be located and appear to have never been submitted; hence measurements not included in the original description were derived from the illustrations provided by Haderlie (1953) of 2 adults using the provided scale bars of (Table 4). *Plagioporus* sp. differs from *P. hageli* n. sp. in having a greater body length (1,230–1,960 μm as opposed to 851–1,198 μm), a shorter forebody (25–26%

of the BL as opposed to 31–38% of the BL), a more anterior position of the caecal bifurcation (19–20% of the BL as opposed to 24–31% of the BL), a wider than long ventral sucker as opposed to one that is subequal, a shorter esophagus (5–8% as opposed to 9–17% of the BL), a submedian ovary that slightly overlaps or is immediately anterior to the testis (n=2) as opposed to one that is dextral to the testis and overlapping it by 2/3 of its length, a smaller ovary (length represents 4–5% compared with 7–8% of the BL) and wider than long as opposed to subequal testes. Haderlie (1953) also reported '*P. angusticolle*' from Boca Lake that although located only *c.* 48 km from the type locality of *P. hageli* n. sp., is part of the Truckee River drainage that flows into Pyramid Lake in Nevada, USA, and is not hydrologically connected to the Sacramento River drainage from which *P. hageli* n. sp. was described. We also could not locate vouchers of '*P. angusticolle*'. Haderlie (1953) did not provide measurements of this species; thus, the measurements in Table 4 were restricted to those derived from the single specimen illustrated. '*Plagioporus angusticolle*' of Haderlie (1953) is very similar to *P. hageli* n. sp., but differs in having a markedly shorter cirrus sac (11% as opposed to 22–28% of the BL), shorter testes (both the anterior and the posterior testis lengths represent only 6% of the BL as opposed to 10–13% of the BL), a larger body size (1,828 μm compared with 851–1,198 μm) and in the possession of distinct median and lateral fields of the vitellarium occupying most of the posttesticular space (in *P. hageli* n. sp. the vitellarium are confluent in this region). Haderlie's (1953) identification of this form is problematic. *Plagioporus angusticollis* (Hausmann, 1896) ('*angusticolle'* is an incorrect spelling) was originally described in Europe by Hausmann (1896) from *Cottus gobio* Linnaeus and subsequent life cycle studies by Mathias (1936, 1937) in Europe indicated that *Anguilla*

anguilla Linnaeus could also serve as a definitive host, with the gastropod *Theodoxus fluviatilis* Linnaeus and peracaridan crustaceans serving as first and second intermediate hosts, respectively. Haderlie (1953) subsequently identified his specimens from California, USA, as '*P. angusticolle*' using the key to *Plagioporus* given by Dobrovolny (1939a), although he noted that his specimens were larger than those from the original description. Manter (1954) detailed further morphological differences, noting a more anterior extent of the vitellarium and a shorter cirrus sac in the specimens of Haderlie (1953). The first intermediate host of *P. angusticollis* is restricted to Europe and Asia, and the family to which it belongs, Neritidae Rafinesque, is restricted to Atlantic Ocean drainages in North America (Turgeon et al., 1998). Given the morphological discrepancies reported by Dobrovolny (1939a) and Manter (1954) and the limited distribution of neritids in North America, we consider any report of *P. angusticollis* in the western Nearctic to represent an undescribed species.

Plagioporus hageli n. sp. is somewhat similar to *Plagioporus hypentelli* Hendrix, 1973 but can be separated from this congener in having an excretory vesicle that is longer (197–269 μm rather than 75–187 μm), a postesticular space that is longer and contains 16-41 as opposed to 3-6 (n=2) vitelline follicles, and vitelline follicles that do not extend to the level of the pharynx. *Plagioporus hageli* n. sp. can be clearly distinguished from all other 7 seven accepted North American species of *Plagioporus* considered valid herein by one or more of the following characteristics: forebody consisting of at least 1/3 of the BL (absent in *P. boleosomi* [Pearse, 1924] Peters, 1957, *P. lepomis* Dobrovolny, 1939), vitellarium in the forebody (absent in *P. boleosomi*, *P. chiliticorum* [Barger & Esch, 1999] Cribb 2005, and *P. lepomis*), posttesticular space greater than 15% BL (absent in

P. serratus Miller, 1940 and *P. sinitsini* Mueller, 1934), and body width representing less than 30% of body length (38-55% of BL in *P. macrouterinus* Haderlie, 1953 and *P. cooperi* [Hunter & Bangham, 1932] Price, 1934, *P. sinitsini*, and *P. serratus*).

Molecular Analysis

No intraspecific variation occurred for sequences obtained from replicates (Table 1) of all 11 species sequenced in this study, including the three replicates of *P. hageli* n. sp. Sequencing reactions for the partial 3' end of the 18S and the entire ITS1 regions were successful only for *P. boleosomi*, *P. hageli* n. sp., *P. kolipinski*, *P. shawi* and *Urorchis acheilognathi* Yamaguti, 1934; therefore, pairwise comparison data of those regions is excluded. Sequence lengths of the complete ITS1 of those five species was 671 bp, 865 bp, 706 bp, 527 bp and 835 bp, respectively. The lengths of the complete 5.8S rDNA sequences were 156 bp for all species, and variability in this region ranged from zero bp between *P. chiliticorum* and *P. kolipinskii*, *Neoplagioporus ayu* (Takahashi, 1928) and *Neoplagioporus zacconis* (Yamaguti, 1934) and *U. acheilognathi* and *Urorchis goro* Ozaki, 1927 to six bp between *P. shawi* and *N. zacconis* and *P. sinitsini* and *N. elongatus* (Goto & Ozaki, 1930).

The alignment used for pairwise comparisons of ITS2 and 28S rDNA sequences was 253 bp and 1,321 bp, respectively. Pairwise comparison of those sequences for newly generated species occur in Table 3. Pairwise comparison of sequences of the ITS2 for all species revealed that *P. hageli* n. sp. was most similar to *P. sinitsini* and *P. chiliticorum*, with respective similarities of 97.6% and 96.0%, whereas sequences of the 28S rDNA gene revealed *P. hageli* n. sp. was most closely related to *Plagiocirrus loboides* (98.6% similar). For both gene regions, species from the Nearctic (*P. loboides*

and species of *Plagioporus*) and the Palearctic (species of *Neoplagioporus* and *Urorchis*) were more similar within than between groups.

The alignment used for BI analysis of partial 28S rDNA sequences was 1,369 characters long. My BI analysis (Fig. 5) resolved a monophyletic Opecoelidae with moderate support. The two species of *Biospeedotrema* Bray, Waeschenbach, Dyal, Littlewood & Morand, 2014 (only representatives of Stenakrinae Yamaguti, 1970) were resolved basal to the rest of the members of the Opecoelidae. Representatives of Helicometrinae Bray, Cribb, Littlewood & Waesenbach, 2016 comprised the clade sister to the remaining opecoelids with strong support. The rest of the opecoelids were separated into two, moderately-supported major clades that in turn were divided into two, strongly-supported subclades. One of the major clades was comprised of 'plagioporines' from shallow-water, marine percomorphs and the two Opistholebetinae Fukui, 1929 representatives. This major clade was divided into two strongly supported subclades: 'Plagioporinae' Clade A (including the Opistholebetinae) and 'Plagioporinae' Clade B similar to the phylogenetic hypothesis presented in Bray et al. (2016). The other major opecoelid clade contained representatives of Opecoelinae Ozaki, 1925 as the strongly supported sister to a clade consisting of deep-sea and freshwater plagioporines as well as the putative opecoelinine *Buticulotrema thermichthysi* Bray, Waeschenbach, Dyal, Littlewood & Morand, 2014. The freshwater plagioporines from the Nearctic (species of *Plagioporus* and *Plagiocirrus loboides*) and the Palearctic (species of *Neoplagioporus* and *Urorchis*) formed a strongly-supported clade that were sister to each other and in-turn, were the strongly-supported sister to the deep-sea species *Bathycreadium brayi* Pérez-del-Olmo, Dallarés, Carrassón & Kostadinova, 2014. This group was in turn sister

to a clade formed by *Allopodocotyle margolisi* Gibson, 1995 and *Gaevskajatrema halosauropsi* Bray & Campbell, 1997 + the putative opecoelinine, and was in turn sister to the early divergent group of this subclade, *Podocotyloides brevis* Andres & Overstreet, 2013 + *Neolebouria lanceolata* (Price, 1934); all of which are from deep-sea fishes and were strongly supported. Within the Palearctic plagioporine clade, *Neoplagioporus ayu* + *N. zacconis* was the strongly-supported sister to *N. elongatus* and *U. acheilognathi* + *U.* goro. Within the Nearctic plagioporine clade, *Plagioporus shawi* was the moderately-supported sister to the other species of *Plagioporus*, within which *Plagiocirrus loboides* was also resolved. Although the clade containing *P. loboides* and the five other species of *Plagioporus* was well supported, the internal relationships were not.

Discussion

The BI analysis based on partial 28S rDNA sequence data resolved plagioporines parasitising freshwater hosts as a monophyletic group within the Opecoelidae, with the Nearctic species from salmonids, gasterosteiids, fundulids, cyprinids, and percids sister to those of the Palearctic from gobiids, plecoglossids and cyprinids. With five morphologically distinct species from salmonids in the Pacific Northwest, including the two forms reported by Haderlie (1953) that likely represent undescribed species and *Plagioporus hageli* n. sp., members of *Plagioporus* have clearly radiated in salmonids in this region. The lack of a close relationship between *P. shawi* and the new species in my BI analysis suggests two independent radiations of *Plagioporus* into salmonids, a finding that is consistent with the considerable host-shifting exhibited by members of this genus noted by Tracey et al. (2009). *Plagioporus hageli* n. sp. was estimated to be most closely related to *Plagiocirrus loboides* rather than *P. shawi* or *P. kolipinskii*, the two congeners

in my analysis also from the western USA. We consider the close relationship of the new species to *Plagiocirrus loboides*, which was described from fundulids and a cyprinid from the Pascagoula River, Mississippi, USA, an artifact of the undersampling of congeners from the western USA. However, my phylogenetic estimation clearly resolves *Plagiocirrus loboides* nestled within *Plagioporus*.

Plagiocirrus Van Cleave & Mueller, 1932 was erected to accommodate *Plagiocirrus primas* Van Cleave & Mueller, 1932 from the intestine of *Notemigonus crysoleucas* (Mitchill) from Oneida Lake, New York, USA. Van Cleave & Mueller (1932) noted the morphological similarity of *Plagiocirrus* to *Plagioporus* but distinguished *Plagiocirrus* from the latter in the possession of a restricted vitelline field immediately posterior to the ventral sucker that encompasses the middle quarters of the body, a uterus that extends posteriorly from the level of the genital pore to the posterior end of the body and an ovary located halfway between the testis and the ventral sucker. Two species of *Plagiocirrus* have subsequently been described; *Plagiocirrus testeus* Fritts, 1959 from the intestine of *Catostomus macrocheilus* Girard from the Clearwater River in Lewiston, Idaho, USA, and *P. loboides*. The ovary of these three species is either situated at the level of the anterior testis (*P. loboides*), immediately pretesticular (*P. testeus*) or well separated from the testis (*P. primas*). Given the variability of the placement of the ovary, only two characters used to originally differentiate members of *Plagiocirrus* from *Plagioporus* remain; a reduced vitelline field and a posteriorly extending uterus. However, both of these characters are represented in members of *Plagioporus*, although not concurrently. The vitelline field of *Plagiocirrus loboides* is restricted to two lateral bands occurring between the anterior margin of the ventral sucker

and the middle of the posterior testis. *Plagioporus chiliticorum* was described from the intestine of *Notropis chiliticus* (Cope) from Basin Creek, North Carolina, USA, and has a similarly reduced vitelline field, with the anterior and the posterior extents occurring at the middle of the ventral sucker and slightly beyond the posterior testis, respectively. Although most species of *Plagioporus* have a uterus that at most extends posteriorly to the anterior margin of the anterior testis, *P. macrouterinus* described from the intestine of *Ptychocheilus oregonensis* (Richardson) from Deer Creek, California, has a uterus that extends as far posteriorly as the middle of the posterior testis. Given the morphological similarity of *Plagiocirrus* to *Plagioporus* and the aforementioned nestling of *Plagiocirrus loboides* within *Plagioporus*, we propose *Plagioporus loboides* comb. n. To accommodate *P. loboides* in *Plagioporus*, the following amendments to the diagnosis of *Plagioporus* are proposed: ovary entire to lobed; uterus between genital pore and posterior end of body; vitelline follicles may enter forebody or may be restricted to hindbody, occasionally restricted to field between ventral sucker and testis. We refrain from making any taxonomic changes to *Plagiocirrus* until sequences from the type species are available; however, given the morphological variability realized in this study for *Plagioporus*, we suspect that *P. primas* and *P. testeus* may belong in *Plagioporus*.

The genetic variability observed within the genera treated in this study is largely consistent with that observed for other opecoelids. Sequences obtained from species of *Plagioporus*, *Neoplagioporus* and *Urorchis* diverged by 1.4–5.0%, 1.6–2.2% and 0.9%, respectively, in the 1,321 bp partial 28S rDNA gene and by 2.4–11.7%, 2.1–4.6% and 1.3%, respectively, in the 253 bp ITS2 rDNA gene (ITS2 data for *P. loboides* comb. n. is lacking). Divergence based on previously published sequences of the partial 28S rDNA

gene between *Opecoeloides furcatus* (Bremser in Rudolpi, 1819) and *O. fimbriatus* (Linton, 1910), *Cainocreadium lintoni* (Siddiqi & Cable, 1960) and *C. labracis* (Dujardin, 1845), and *Macvicaria obovata* (Molin, 1859), *M. maamouriae* Antar, Georgieva, Gargouri & Kostadinova, 2015, *M. dubia* (Stossich, 1905), *M crassigula* (Linton, 1910), *M. mormyri* (Stossich, 1885) and *M. bartolii* Antar, Georgieva, Gargouri & Kostadinova, 2015 was 4% (of 1,235 bp), 4 % (of 1,284 bp) and 0.6–2.2% (of 1,176 bp), respectively. While intrageneric ITS2 rDNA sequence variability is largely lacking for most opecoelid genera with sequence data, the six previous species of *Macvicaria* plus *M. mallairdi* Bartoli, Bray & Gibson, 1989 diverge by 0.2–2.5% in 255 bp of the ITS rDNA gene. The higher sequence divergences observed for species of *Plagioporus* compared with other opecoelid genera was driven by *P. shawi* and varied from the other species of *Plagioporus* by 3.7–5.0% in the 28S and 9.6–11.7% in the ITS2.

Gibson & Bray (1982) restricted *Plagioporus* to freshwater forms with an excretory vesicle extending anteriorly to at most the level of the posterior testis, a diagnosis that Cribb (2005) followed. However, *P. shawi* and *P. siliculus* have a long excretory vesicle that extends beyond the posterior testis and both were retained within *Plagioporus* by Cribb (2005). Additionally, *Allopodocotyle virens* (Sinitsin, 1931) Pritchard, 1966, which was originally described from the intestine of a freshwater sculpin *Cottus* sp. from Oregon, USA, as *Plagioporus virens* Sinitsin, 1931, has been retained in *Allopodocotyle* Pritchard, 1966 despite the restriction of this genus to marine hosts, presumably because *A. virens* possesses a long excretory vesicle and vitelline follicles restricted to the hindbody. However, we suspect that *P. shawi* is closely related to *A. virens* because both species have an excretory vesicle that extends to at least the level of

the ovary and the life cycles of both species occur in freshwater habitats and use lithoglyphid first intermediate hosts in the genus *Fluminicola* Stimpson (Schell 1975; Sinitsin 1931). Apart from *P. shawi*, all other species of *Plagioporus* in the Nearctic with a known life cycle (four species) have cerithioidean first intermediate hosts of the families Pleuroceridae Fischer or Semisulcospiridae Morrison (Sinitsin 1931; Dobrovolny 1939a, 1939b; Hendrix 1978). If my suspicion is correct, a separate genus will likely be necessary to accommodate at least *P. shawi* and *A. virens* based on where *P. shawi* was resolved within my BI analysis (Figure 5). However, I currently refrain from making any taxonomic decisions until sequence data are available for *A. virens* or other species of *Plagioporus* with long excretory vesicles because of the amount of homoplasy observed with most opecoelid characters.

Bray et al. (2016) stated that the excretory vesicle length appears to be an informative character. If so, perhaps *Plagiocirrus* could accommodate *Plagioporus shawi*, *P. siliculus* and *A. virens* because *Plagiocirrus primas* also possesses a long excretory vesicle (Van Cleave & Mueller, 1934); however, *P. primas* could also be resolved with the other species of *Plagioporus* similar to *P. loboides*. Conversely, *Nezpercella* Schell, 1974 may accommodate *P. shawi* and *A. virens* despite members of that genus having a short excretory vesicle because the type species, *Nezpercella lewisi* Schell, 1974 also uses lithoglyphid snails as the first intermediate host (Schell, 1976). The possession of a long excretory vesicle is also seen in some freshwater plagioporines from Japan; the excretory vesicle of *U. goro*, *U. acheilognathi* and *Urorchis imba* Ishii, 1935 extends to the middle of the anterior testis, to the anterior margin of the anterior testis, and to the level of the ovary, respectively (Shimazu, 1990a), and that of *N.*

zacconis and *N. elongatus* extends anteriorly to the level of the anterior testis (Shimazu, 1990b). All of those species with the exception of *U. imba* are represented in my BI analysis, with *N. ayu* being the only Palearctic freshwater plagioporine included in the analysis with an excretory vesicle not reaching the level of the anterior testis (maximum anterior extent at middle of posterior testis). Thus, the length of the excretory vesicle may not necessarily be useful in distinguishing freshwater plagioporine genera from marine 'plagioporine' genera with which they are often confused. *Neoplagioporus* is not well distinguished from *Urorchis*; the only non-overlapping characters that can be used to separate members of these genera are the posterior extent of the uterus and embryonation of the eggs. Species of *Neoplagioporus* have a uterus containing unembryonated eggs that is usually pretesticular but can extend posteriorly to the middle of the posterior testis whereas species of *Urorchis* have a uterus that contains embryonated eggs and has loops that extend to the posterior end of the body (Shimazu 1990a, 1990b). My BI analysis could not resolve the placement of the placement of *N. elongatus.* It was slightly more related to the two species of *Urorchis* than it was to the other two species of *Neoplagioporus*. Pairwise comparison of the ITS2 rDNA sequence data (Table 3) also suggested a close association of *N. elongatus* with two species of *Urorchis*. Given that the posterior extent of the uterus may not be a useful character in distinguishing opecoelid genera (e.g. *Plagiocirrus*) and the unresolved placement of *N. elongatus*, we could be justified in reducing *Neoplagioporus* to a junior synonym of *Urorchis*. However, we await molecular data for additional species of *Urorchis* and *Neoplagioporus* before making any taxonomic changes.

The degree of morphological variation realized in this study for *Plagioporus* will make difficult the morphological distinction of this genus from other marine and freshwater opecoelid genera. Biogeography, host identity and molecular data are all useful supplements to morphological distinction in the case of *Plagioporus*. My BI analysis found that the most closely related opecoelids to *Plagioporus* are species of *Urorchis* and *Neoplagioporus*. While the distinguishing morphological features between these Palearctic and Nearctic genera are few, *Urorchis* and *Neoplagioporus* are collectively distinguished from *Plagioporus* in the possession of a contiguously bipartite seminal vesicle (Shimazu, 1990a, 1990b). While *P. chiliticorum* has a bipartite seminal vesicle, its seminal vesicle is not contiguously bipartite as the chambers of the seminal vesicle are separated by a distinct duct (Barger & Esch, 1999). This distinguishing feature may not be useful if *Pseudurorchis catostomi* Schell, 1974, which possesses a contiguously bipartite seminal vesicle and was described from *C. macrocheilus* from the Clearwater River in Idaho, USA, proves to be a species of *Plagioporus*. Schell (1974) may have created an unnatural group in assigning this species to *Pseudurorchis* Yamaguti, 1971, which at the time contained two species from freshwater fish in Israel (Schell, 1974). Given that *Plagioporus* was erected for a Nearctic freshwater fish and is phylogenetically distinct from Palearctic freshwater plagioporines from which it is not clearly distinguished morphologically, it is possible that *Plagioporus* represents a Nearctic genus. We recommend the inclusion of species assigned to *Plagioporus* from the Palearctic in future studies to clarify the biogeography of plagioporine genera parasitizing freshwater hosts.

My BI analysis was mostly consistent with that of the opecoelid phylogeny produced by Bray et al. (2016) but with lower support for the monophyly of the Opecoelidae, likely because we strictly analysed partial 28S rDNA sequence data (see Bray et al. 2016). My BI analysis represents the first phylogenetic study of the opecoelids to include the type genus of Plagioporinae, *Plagioporus*, and helped to clarify the phylogenetic position of this subfamily. My analysis also confirmed the suggestion by Bray et al. (2016) that species of *Plagioporus* would likely be resolved near *P. loboides*. A clade containing freshwater + deep-sea plagioporines was demonstrated in previous phylogenies (Andres et al., 2014a; Shedko et al., 2015; Bray et al., 2016) and resolved sister to the representative opecoelines; however, that clade was considered incerate sedis by Shedko et al. (2015) and 'Plagioporinae' by Bray et al. (2016). Presumably, those authors did so because the vast majority of other plagioporine-like taxa were resolved in a separate, strongly-supported clade. Additionally, *Buticulotrema thermichthysi*, the lone putative representative of Opecoelininae Gibson & Bray 1984 with sequence data, was resolved sister to *Gaevskajatrema halosauropsi* Bray & Campbell, 1996 (as it was in m analysis) complicating the systematics of the freshwater + deep-sea clade. I believe that restricting the Plagioporinae to only *Plagioporus*-like freshwater forms would be premature because *Bathycreadium brayi* was the strongly-supported sister to the freshwater plagioporines, and doing so would necessitate at least three other subfamilies for the non-stenakrine deep-sea opecoelids. My analysis further echoes what Cribb (2005) and other authors have suggested in that opecoelid subfamilial classification is complex and unsatisfactory.

Based on the limited representative sequences that are available, a potential pattern related to Plagioporinae sensu lato taxa and their final hosts may be discerned. The taxa I consider to be plagioporines (Figure 5) tend to parasitise basal (non-percomorph) actinopterygian fishes, and three out of the four species that do have percomorph final hosts (*P. boleosomi*, *P. kolipinskii* and *U. goro*) are likely the result of host-switching events in freshwater because each of those species were resolved on internal branches. All representatives of 'Plagioporinae' Clades A and B parasitise marine percomorph fishes that are predominantly found in shallow-water (continental shelf and shallower) habitats. While I agree with previous authors (e.g., Curran et al., 2007; Andres et al., 2014a; Bray et al. 2014, 2016; Shedko et al., 2015) that additional representative opecoelids are necessary before making major changes, I believe that *Buticulotrema* likely belongs in the Plagioporinae. Although both species of this genus possess a reduced cirrus sac, Bray et al. (2016) suggested that a reduced cirrus sac was potentially a homoplasious character. *Buticulotrema stenauchenus* Blend, Dronen, & McEachran, 1993 (the type species of the genus) parasitise deep-sea, non-percomorph fish, but the type species of Opecoelininae, *Opecoelina scorpaenae* Manter, 1934, parasitises a shallow-water, marine percomorph that may indicate the opecoelinines have a closer affinity to the marine percomorph opecoelid clade. Complicating my consideration, *B. thermichthysi* was the only marine species included in my BI analysis that was resolved in the Plagioporinae clade (Fig. 5) that parasitises a percomorph. However, its host is an ophidiiform; the order that represents the most basal percomorph order (see Near et al., 2012), and is unique in that the Ophidiiformes have reached their

greatest diversity in deep-sea and tropical reef habitats (Møller et al., 2016). Therefore, this host could also be the result of a host switching event.

I note that the subfamily Urorchiinae Shimazu, 1990 might not be a phylogenetically useful grouping. Urorchiinae united genera of opecoelids parasitising freshwater fishes with a uterus extending to the hindbody and possessing a bipartite seminal vesicle. Shimazu (1990a) included the genera *Urorchis*, *Pseudurorchis* and *Plagiocirrus* in this subfamily, although the later was shown not to possess a bipartite seminal vesicle (Curran et al., 2007). Cribb (2005) suggested that *Nezpercella* Schell, 1974 and *Multivitellina* Schell, 1974 correspond well with Shimazu's (1990a) concept of the subfamily, but these genera do not possess a bipartite seminal vesicle. In my analysis, *P. loboides* and *Urorchis* were more closely related to *Plagioporus* and *Neoplagioporus*, respectively, than they were to one another. My molecular hypothesis establishes the placement of the Plagioporinae sensu stricto, and further demonstrates the evolutionary complexity exhibited within the family.

Table 1 Species of Opecoelidae collected from the Holarctic and their respective hosts, collection localities, collection years, GenBank accession number (with number of replicates in parenthesis) and deposition information.

Species	Host	Collection locality	Year	GenBank No.	NMNH
Neoplagioporus ayu (Takahashi, 1928)	*Plecoglossus altivelis altivelis* (Temminck & Schlegel)	Asahi River, Okayama City, Okayama Prefecture, Japan	2013	KX553947 (2)	1416796
Neoplagioporus elongatus (Goto & Ozaki, 1930)	*Sarcocheilichthys variegatus microoculus* Mori	Lake Biwa, Takashima City, Shiga Prefecture, Japan	2012	KX553948 (3)	1416795
Neoplagioporus zacconis (Yamaguti, 1934)	*Opsariichthys platypus* (Temminch & Schlegel)	Uji River, Uji City, Kyoto Prefecture, Japan	2012	KX553949 (2)	1416794
Plagioporus boleosomi (Pearse, 1924)	*Percina maculata* (Girard)	West Twin River, Wisconsin, USA	2009	KX553953 (3)	1416789
Plagioporus chiliticorum (Barger & Esch, 1999)	*Notropis chiliticus* (Cope)	Basin Creek, North Carolina, USA	2012	KX553943 (2)	1416791
Plagioporuis hageli sp. nov.	*Oncorhynchus mykiss* (Walbaum)	Yuba River, California, USA	2010	KX553950 (3)	1416782-5
Plagioporus kolipinskii Tracey, Choudhury, Cheng & Ghosh 2009	*Gasterosteus aculeatus* Linnaeus	Lobos Creek, California, USA	2009	KX553952 (3)	1416787
Plagioporus shawi (McIntosh, 1939)	*Oncorhynchus tshawytscha* (Walbaum)	McKenzie River, Oregon, USA	2011	KX553951 (3)	1416790
Plagioporus sinitsini Mueller, 1934	*Notemigonus crysoleucas* (Mitchill)	St. Lawrence River, Montreal, Canada	2013	KX553944 (3)	1416786
Urorchis acheiloghathi Yamaguti, 1934	*Tanakia limbata* (Temminck & Schlegel)	Irrigation canal at Nishiyama, Nagahama City, Shiga Prefecture, Japan	2013	KX553945 (2)	1416793
Urorchis goro Ozaki, 1927	*Rhinogobius* sp.	Small stream at Oomura, Matsumoto City, Nagano Prefecture, Japan	2013	KX553946 (2)	1416792

Table 2 Sequences obtained from GenBank used for phylogenetic analysis

Family	Species	Host	GenBank No.	Reference
Brachycladiidae	*Zalophotrema hepaticum* Stunkard & Alvey, 1929	*Zalophus californianus* (Lesson)	AY222255	Olson et al. (2003)
Acanthocolpidae	*Stephanostomum pristis* (Deslongchamps, 1824)	*Phycis phycis* (Linnaeus)	DQ248222	Bray et al. (2005)
Enenteridae	*Enenterum aurem* Linton, 1910	*Kyphosus vaigiensis* (Quoy & Gaimard)	AY222232	Olson et al. (2003)
Lepocreadiidae	*Preptetos caballeroi* Pritchard, 1960	*Naso vlamingii* (Valenciennes)	AY222236	Olson et al. (2003)
Opecoelidae	*Allopodocotyle epinepheli* (Yamaguti, 1942)	*Epinephelus cyanopodus* (Richardson)	KU320598	Bray et al. (2016)
Opecoelidae	*Allopodocotyle margolisi* Gibson, 1995	*Coryphaenoides mediterraneus* (Giglioli)	KU320596	Bray et al. (2016)
Opecoelidae	*Allopodocotyle* sp. A	*Scolopsis bilineata* (Bloch)	KU320599	Bray et al. (2016)
Opecoelidae	*Allopodocotyle* sp. B	*Epinephelus coioides* (Hamilton)	KU320607	Bray et al. (2016)
Opecoelidae	*Anomalotrema koiae* Gibson & Bray, 1984	*Sebastes viviparus* Krøyer	KU320595	Bray et al. (2016)
Opecoelidae	*Bathycreadium brayi* Pérez-del-Olmo, Dallarés, Carrassón & Kostadinova, 2014	*Trachyrincus scabrus* (Rafinesque)	JN085948	Constenla et al. (2011)
Opecoelidae	*Bentholebouria blatta* (Bray & Justine, 2009)	*Pristipomoides argyrogrammicus* (Valenciennes)	KU320606; KU320608	Bray et al. (2016)
Opecoelidae	*Bentholebouria colubrosa* Andres, Pulis & Overstreet 2014	*Pristipomoides aquilonaris* (Goode & Bean)	KJ001207	Andres et al. (2014a)
Opecoelidae	*Biospeedotrema biospeedoi* Bray, Waeschenbach, Dyal, Littlewood & Morand (2014)	*Thermichthys hollisi* (Cohen, Rosenblatt & Moser)	KF733986	Bray et al. (2014)

Opecoelidae	*Biospeedotrema jolliveti* Bray, Waeschenbach, Dyal, Littlewood & Morand (2014)	*Ventichthys biospeedoi* Nielsen, Møller & Segonzac	KF733985	Bray et al. (2014)
Opecoelidae	*Buticulotrema thermichthysi* Bray, Waeschenbach, Dyal, Littlewood & Morand , 2014	*Thermichthys hollisi* (Cohen, Rosenblatt & Moser)	KF733984	Bray et al. (2014)
Opecoelidae	*Cainocreadium labracis* (Dujardin, 1845)	*Gibbula adansonii* (Payraudeau)	JQ694144	Born-Torrijos et al. (2012)
Opecoelidae	*Cainocreadium lintoni* (Siddiqi & Cable, 1960)	*Epinephelus morio* (Valenciennes)	KJ001208	Andres et al. (2014a)
Opecoelidae	*Dimerosaccus oncorhynchi* (Eguchi, 1931)	*Oncorhynchus masou* (Brevoort)	FR870252	Shedko et al. (2015)
Opecoelidae	*Gaevskajatrema halosauropsi* Bray & Campbell, 1996	*Halosauropsis macrochir* (Günther)	AY222207	Olson et al. (2003)
Opecoelidae	*Gaevskajtrema perezi* (Mathias, 1926)	Unidentified fish host	AF184255	Tkach et al. (2001)
Opecoelidae	*Hamacreadium mutabile* Linton, 1910	*Lutjanus griseus* (Linnaeus)	KJ001209	Andres et al. (2014a)
Opecoelidae	*Hamacreadium 'mutabile'*	*Lutjanus fulviflamma* (Forsskål)	KU320601	Bray et al. (2016)
Opecoelidae	*Hamacreadium* sp.	*Lethrinus miniatus* (Forster)	KU320603	Bray et al. (2016)
Opecoelidae	*Helicometra boseli* Nagaty, 1956	*Sargocentron spiniferum* (Forsskål)	KU320600	Bray et al. (2016)
Opecoelidae	*Helicometra epinepheli* Yamaguti, 1934	*Epinephelus fasciatus* (Forsskål)	KU320597	Bray et al. (2016)
Opecoelidae	*Helicometra manteri* Andres, Ray, Pulis, Curran & Overstreet, 2014	*Prionotus alatus* Goode & Bean	KJ701238	Andres et al. (2014b)
Opecoelidae	*H. manteri*	*Bellator egretta* (Goode & Bean)	KJ701239	Andres et al. (2014b)
Opecoelidae	*Maculifer* sp.	*Diodon hystrix* Linnaeus	AY222211	Olson et al. (2003)
Opecoelidae	*Macvicaria bartolii* Antar, Georgieva, Gargouri & Kostadinova, 2015	*Diplodus annularis* (Linnaeus)	KR149464	Antar et al. (2015)
Opecoelidae	*Macvicaria crassigula* (Linton, 1910)	*Calamus bajonado* (Black & Schneider)	KJ701237	Andres et al. (2014b)
Opecoelidae	*Macvicaria dubia* (Stossich, 1905)	*Oblada melanura* (Linnaeus)	KR149469	Antar et al. (2015)

Opecoelidae	*Macvicaria macassarensis* (Yamaguti, 1952)	*Lethrinus miniatus* (Forster)	AY222208	Olson et al. (2003)
Opecoelidae	*Macvicaria mormyri* (Stossish, 1885)	Unidentified fish host	AF184256	Tkach et al. (2001)
Opecoelidae	*Macvicaria obovata* (Molin, 1859)	*Cyclope neritea* (Linnaeus)	JQ694147	Born-Torrijos et al. (2012)
Opecoelidae	*Neolebouria lanceolata* Andres, Pulis & Overstreet, 2014	*Polymixia lowei* (Günther)	KJ001210	Andres et al. (2014a)
Opecoelidae	*Opecoeloides fimbriatus* (Linton, 1910)	*Micropogonias undulatus* (Linnaeus)	KJ001211	Andres et al. (2014a)
Opecoelidae	*Opecoeloides furcatus* (Bremser in Rudolphi, 1819)	*Mullus surmuletus* Linnaeus	AF151937	Tkach et al. (2000)
Opecoelidae	*Opistholebes amplicoelus* Nicoll, 1915	*Tetractenos hamiltoni* (Richardson)	AY222210	Olson et al. (2003)
Opecoelidae	*Pacificreadium serrani* (Nagaty & Abdel-Aal, 1962)	*Plectropomus leopardus* (Lacepède)	KU320602	Bray et al. (2016)
Opecoelidae	*Peracreadium idoneum* (Nicoll, 1909)	*Anarhichas lupus* Linnaeus	AY222209	Olson et al. (2003)
Opecoelidae	*Plagiocirrus loboides* Curran, Overstreet & Tkach, 2007	*Fundulus nottii* (Agassiz)	EF523477	Curran et al. (2007)
Opecoelidae	*Podocotyloides brevis* Andres & Overstreet, 2013	*Conger esculentus* Poey	KJ001212	Andres et al. (2014a)
Opecoelidae	*Pseudopecoeloides tenuis* Yamaguti, 1940	*Priacanthus hamrur* (Forsskål)	KU320605	Bray et al. (2016)
Opecoelidae	*Pseudopycnadena tendu* Bray & Justine, 2007	*Pseudobalistes fuscus* (Bloch & Schneider)	FJ788506	Bray et al. (2009)
Opecoelidae	*Propycnadenoides philippinensis* Fischthal & Kuntz, 1964	*Gymnocranius grandoculis* (Valenciennes)	KU320604	Bray et al. (2016)

Table 3 Pairwise comparisons of percent nucleotide similarity and the number of base pair differences (in parentheses) for the ITS-2 (below the diagonal) and 28S (above the diagonal) of *Plagiocirrus loboides* (28S only; EF523477) and species of *Plagioporus*, *Neoplagioporus* and *Urorchis* provided in this study.

	Plagiocirrus loboides	*Plagioporus boleosomi*	*Plagioporus chiliticorum*	*Plagioporus hageli* n. sp.	*Plagioporus kolipinskii*	*Plagioporus sinitsini*	*Plagioporus shawi*	*Neoplagioporus ayu*	*Neoplagioporus zacconis*	*Neoplagioporus elongatus*	*Urorchis acheilognathi*	*Urorchis goro*
Plagiocirrus loboides	–	98.1 (22)	97.7 (27)	98.6 (16)	97.2 (32)	97.7 (27)	96.3 (42)	93.9 (70)	93.9 (70)	94.5 (64)	94.0 (69)	94.4 (65)
Plagioporus boleosomi	NA	–	97.3 (35)	97.4 (34)	96.7 (43)	97.5 (33)	96.2 (50)	93.5 (85)	93.4 (87)	94.0 (79)	93.8 (81)	94.0 (79)
Plagioporus chiliticorum	NA	94.0 (15)	–	97.2 (37)	96.3 (49)	97.7 (30)	95.6 (58)	93.5 (86)	93.4 (87)	94.1 (78)	93.9 (80)	94.0 (79)
Plagioporus hageli n. sp.	NA	95.6 (11)	96.0 (10)	–	96.8 (42)	97.6 (32)	96.2 (50)	94.2 (77)	93.9 (81)	94.5 (73)	94.2 (76)	94.5 (72)
Plagioporus kolipinskii	NA	96.0 (10)	93.5 (16)	94.8 (13)	–	96.6 (45)	95.0 (65)	93.3 (88)	93.3 (88)	93.4 (87)	93.2 (90)	93.5 (86)
Plagioporus sinitsini	NA	96.8 (8)	96.0 (10)	97.6 (6)	95.6 (11)	–	95.9 (53)	94.2 (76)	94.0 (79)	94.7 (70)	94.5 (72)	94.8 (68)
Plagioporus shawi	NA	90.4 (23)	88.3 (28)	90.4 (23)	89.9 (24)	90.0 (24)	–	94.7 (69)	94.9 (67)	95.7 (56)	95.2 (62)	95.4 (60)
Neoplagioporus ayu	NA	87.9 (29)	84.9 (36)	86.6 (32)	85.7 (34)	86.2 (33)	88.3 (28)	–	98.4 (21)	97.9 (28)	97.3 (35)	97.6 (32)
Neoplagioporus zacconis	NA	87.9 (29)	86.6 (32)	89.1 (26)	86.5 (32)	88.7 (27)	88.3 (28)	95.4 (11)	–	97.8 (29)	97.3 (35)	97.3 (35)
Neoplagioporus elongatus	NA	87.4 (30)	86.2 (33)	88.7 (27)	86.5 (32)	87.4 (30)	87.4 (30)	95.8 (10)	97.9 (5)	–	98.9 (15)	99.2 (11)
Urorchis acheilognathi	NA	86.2 (33)	85.8 (34)	88.3 (28)	85.7 (34)	87.0 (31)	86.2 (33)	93.7 (15)	96.7 (8)	97.9 (5)	–	99.1 (12)
Urorchis goro	NA	87.4 (30)	87.0 (31)	89.5 (25)	86.5 (32)	88.3 (28)	87.4 (30)	94.1 (14)	97.1 (7)	98.3 (4)	98.7 (3)	–

Table 4 Dimensions and ratios of species of *Plagioporus* from *Oncorhynchus mykiss* in California including *Plagioporus* sp. and '*Plagioporus angusticolle*' of Haderlie (1953), and *Plagioporus hageli* n. sp.

	Plagioporus sp. (n = 1, 2, or 5)	*'Plagioporus angusticolle'* (n = 1)	*Plagioporus hageli* n. sp. (n = 10)
Body length (BL)	1,230–1,960*; 1,778, 1,1812 †	1,828 †	851–1,198
Body width (BW)	410–610*; 481, 611 †	409 †	232–313
BL:BW	1:0.27, 1:0.34 †	1:0.22 †	1:0.26–0.28
Oral sucker length as % BL	11, 13 †	10 †	8–11
Ventral sucker length as % BL	13, 14 †	12 †	13–16
Ventral sucker width as % BW	75, 55 †	61 †	61–69
Width of OS:VS	1:1.7, 1:1.5 †	1:1.4 †	1:1.5–1.8
Pharnyx length as % BL	8, 7 †	6 †	5–6
Oesophagus length as % BL	8, 5 †	12 †	9–17
Intestinal bifurcation as % BL	20, 19 †	29 †	24–31
Post-caecal space as % BL	5, 7 †	5 †	7–13
Forebody as % BL	26, 25 †	36 †	31–38
Anterior testis length as % BL	9, 8 †	6 †	10–12
Anterior testis position as % BL	57, 58 †	67 †	55–63
Posterior testis length as % BL	10, 10 †	6 †	11–13
Posterior testis position as % BL	65, 66 †	74 †	65–73
Posttesticular space as % BL	24, 24 †	21 †	19–22
Cirrus-sac length as % BL	23 †	11 †	22–28
Genital pore position as % BL	18 †	27 †	22–26
Ovary length as % BL	4, 5 †	7 †	7–8
Ovary position as % BL	53, 54 †	60 †	55–59
Postovarian space as % BL	42, 41 †	33 †	34–39
Egg length	59–63*; 49, 53 †	54 †	56–69
Egg width	35–39*; 24, 32 †	28 †	31–41
Anterior extent of vitellarium as % BL	19, 22 †	27 †	22–31

* Measurements reported by Haderlie (1953) (n = 5)

† Measurements derived from the line drawings of Haderlie (1953) using provided scale-bar (n =1–2)

Figure 1. Plagioporus hageli n. sp. from the intestine of *Oncorhynchus mykiss*. 1, Ventral view of holotype; 2, Dorsal view of paratype; 3, Dorsal view of female complex; 4, Ventral view of cirrus sac and metraterm. *Scale bars* Figs1-2: 100 µm *Scale bars* Figs 3-4: 50 µm

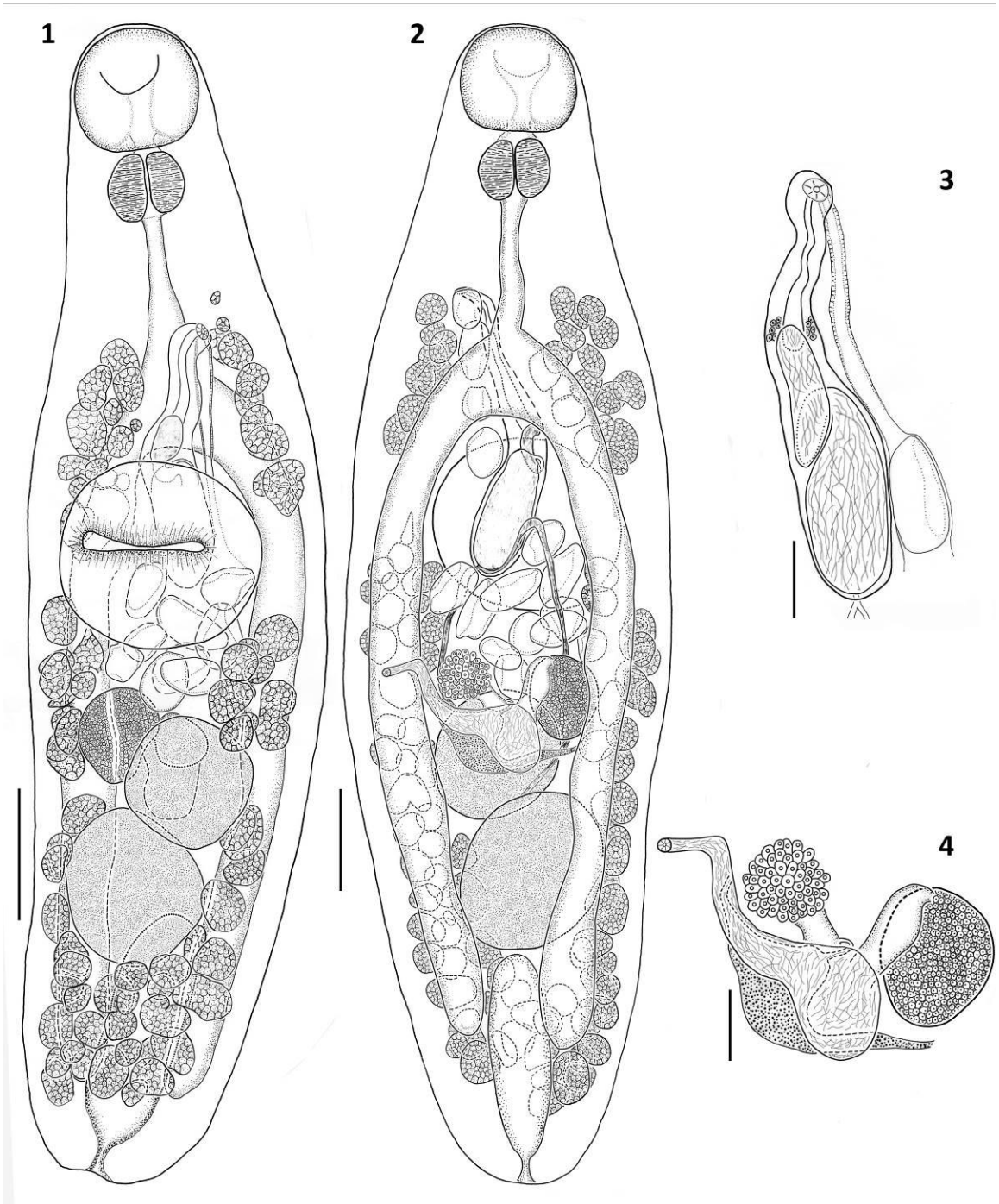
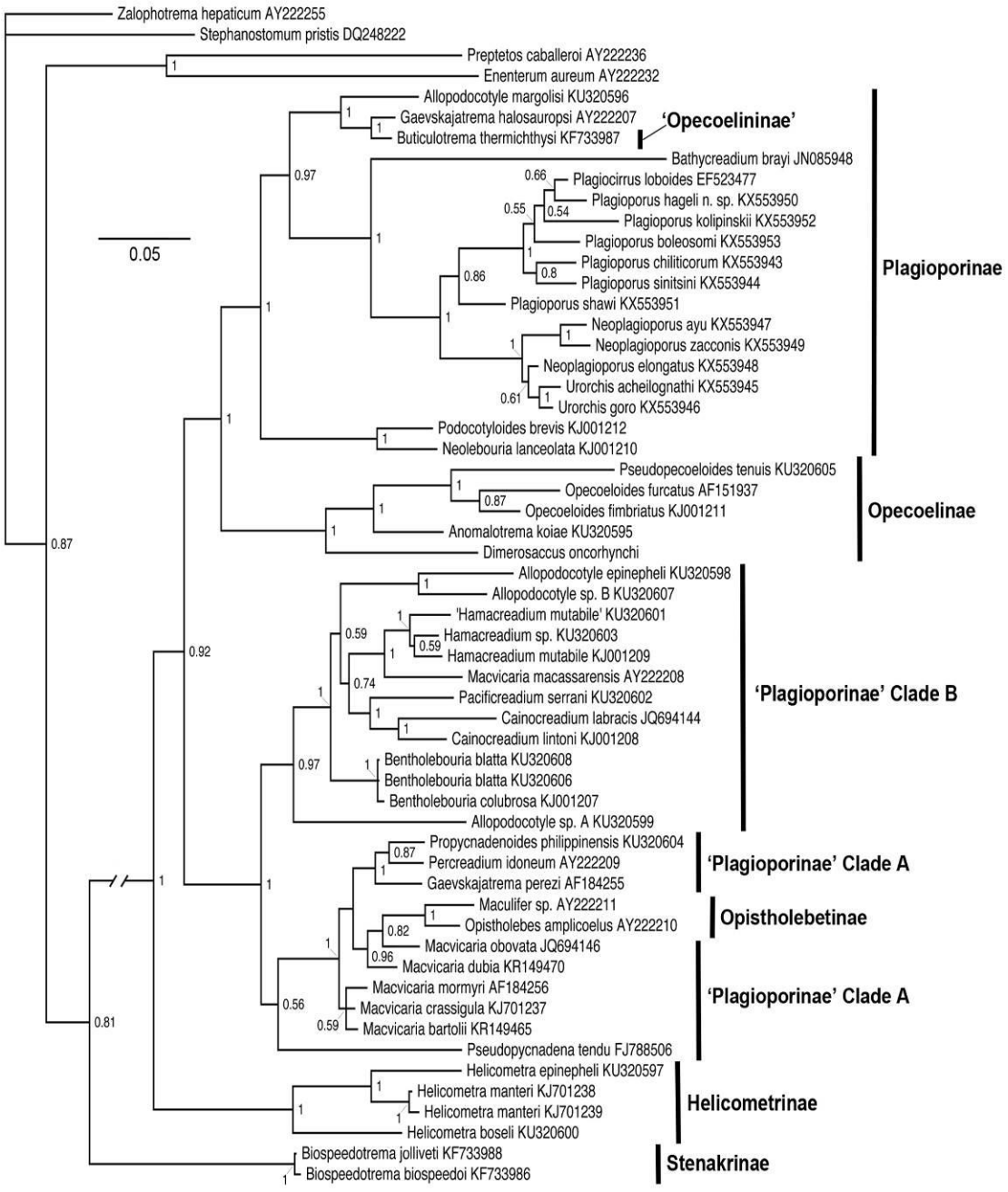


Figure 2. Phylogenetic relationships among members of the Opecoelidae resulting from Bayesian inference analysis of partial 28S rDNA sequences (GTR + I + Γ) (5,000,000 generations and a sample frequency of 1,000). The length of the truncated branch is 0.09.



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CHAPTER III - Two new species of *Plagioporus* Stafford, 1904 from the Ouachita Madtom, *Noturus lachneri* Taylor, and the Banded Sculpin, *Cottus carolinae* (Gill), from Arkansas, U.S.A.

Abstract

Plagioporus ictaluri n. sp. and *Plagioporus carolini* n. sp. are described from the intestine of the Ouachita Madtom, *Noturus lachneri* Taylor, from the Ouachita River drainage and the Banded Sculpin, *Cottus carolinae* (Gill), from the Arkansas River drainage, respectively, from Arkansas, U.S.A. The new species are morphologically most similar to one another and in turn similar to *Plagioporus sinitsini* Mueller, 1934, *Plagioporus chilitcorum* (Barger & Esch, 1999) Cribb, 2005 , *Plagioporus serratus* Miller, 1940 and *Plagioporus hypentelii* Hendrix, 1973, but can be distinguished from these congeners in possession of an excretory vesicle that extends anteriorly to the level of the anterior testis as opposed to one only reaching the posterior testis (*P. hypentelii*) or one confined to the posttesticular space (*P. sinitsini*, *P. serratus* and *P. chiliticorum*), a feature that necessitates altering the generic diagnosis for the genus. *Plagioporus carolini* n. sp. is distinguished from *Plagioporus ictaluri* n. sp. in having oblique versus tandem testis, an excretory vesicle with a maximum anterior extent at the level of the ovary versus one only reaching the level of the anterior testis, a dextral ovary as opposed to one that is submedian to median, a ventral sucker occupying 80-92% of the body width (BW) versus 53-71% BW, an oral sucker occupying 49-58% of the body width as opposed to 36-47% and a pharynx occupying 28-36% BW compared to 21-26% BW. A Bayesian inference (BI) analysis of partial 28S rDNA sequences of the 2 new species and those of 24 opecoelids obtained from GenBank was conducted to estimate the new species

placement within *Plagioporus* Stafford, 1904. This BI analysis not only confirmed that the 2 new species are more closely related to one another than to other congeners but also resolved *P. chiliticorum* as sister to the new species, forming a clade that was in turn sister to *P. sinitsini*. The interrelationships between *Plagioporus ictaluri* n. sp., *Plagioporus carolini* n. sp., *P. chiliticorum* and *P. sinitsini* from the BI analysis and the morphological comparison are reflected by pairwise comparisons of the ITS rDNA region, and these 4 species are notably the only Nearctic plagioporids without a uterus extending to the posterior end that lack a confluent vitelline field in the posttesticular space (excluding *P. serratus*, for which sequence data is not available). This study includes the first species of *Plagioporus* to be described from an ictalurid host and the first species in the genus to be described from a cottid east of the Rocky Mountains.

Introduction

In the Nearctic region, *Plagioporus* Stafford, 1904 consists of 14 species parasitizing freshwater and diadromous fishes, including species described from the intestine of cyprinids, catostomids, percids, salmonids, gasterosteids, fundulids and centrarchids and those from the gall bladder of cyprinids, hiodontids and catostomids. Notably absent from this list of type hosts are ictalurids and cottids (Hoffman, 1999; Fayton & Andres, 2016) despite reports from these hosts (Bangham, 1951; Harms, 1959, 1960; McAllister et al., 2014a, 2014b, 2015). Harms (1959, 1960) reported *Plagioporus* sp. from the intestine of the Black Bullhead, *Ameiurus melas* (Rafinesque), from Northeastern Kansas, but did not specify the county. In Arkansas, McAllister et al., (2014a) reported *Plagioporus* sp. from the Ouachita Madtom, *Noturus lachneri* Taylor, from the Middle Branch of Gulpha Creek in the upper Ouachita River drainage.

Subsequently, McAllister et al., (2015) reported *Plagioporus* sp. from the intestine of an additional madtom species, the Slender Madtom, *Noturus exilis* Nelson, from Flint Creek, Arkansas, and noted that they could not distinguish their specimens morphologically from *Plagioporus* sp. from *Cottus carolinae* (Gill) reported by McAllister et al., (2014b) also collected from Flint Creek, Arkansas. An additional form, *Allopodocotyle virens* (Sinitsin, 1931) Pritchard, 1966, from *Cottus* sp. described by Sinitsin (1931) from the Siuslaw River upstream of river mile 17, Oregon, was described as a member of *Plagioporus* (Sinitsin, 1931), but is currently retained *Allopodocotyle* Pritchard, 1966 sensu Cribb (2005) despite the stated restriction of this genus to marine hosts. *Plagioporus* has also been reported from the Mottled Sculpin, *Cottus bairdii semiscaber* (Cope) (reported as *Cottus semiscaber*) from Wyoming (Bangham, 1951). Though Bangham (1951) identified these specimens as *Plagioporus cooperi* (Hunter & Bangham, 1932) Price 1934, a species described from cyprinids from Lake Erie and subsequently reported from other species of cyprinids and darters from Mississippi River drainages eastwards (Hoffman, 1999), this is the only report of this species from both a cottid host and from western North America.

I collected *Plagioporus* sp. from *C. carolinae* from the same Benton County localities in the Arkansas River drainage of Arkansas as did McAllister et al. (2014b). Additionally, I collected a morphologically similar, but distinct congener from *Noturus lachneri*, which is endemic to the upper Ouachita River drainage in Arkansas, at the same site sampled by McAllister et al. (2014a). I use morphological and molecular methods to describe the Flint Creek and upper Ouachita River forms as new species and use

ribosomal rRNA gene sequence data to assess the phylogenetic relationships of these new species with their congeners.

Material and Methods

On 19 March, 2014, specimens of *Plagioporus* were collected from Flint Creek off Fairmont Road at Springtown, Benton County, Arkansas (36º 15' 9.9" N, 94º 26' 25.8" W), and at Flint Creek south of Gentry off US 59, Benton County, Arkansas (36º 14' 33.8" N, 94º 29' 14.5" W), from the intestine of *C. carolinae* via kicknet. On 26 November, 2014, specimens of *Plagioporus* were collected from *N. lachneri* from Middle Branch of Gulpha Creek off East Grand Avenue, Hot Springs, Garland County, Arkansas (34° 30' 33.17" N, 93 ° 00' 32.48" W). Specimens of opecoelids were removed from the intestine of fish hosts with the aid of a fine paintbrush and transferred to and observed in a shallow dish containing 0.6% saline. Subsequently, most of the saline was removed from this dish to the point where worms were restricted to the surface of the dish and attached to the glass by their suckers, upon which near boiling (steaming hot) water was rapidly added to kill worms, minimizing contraction or curling post-fixation. Heat-killed worms were immediately transferred to 10% neutral phosphate buffered formalin for morphological examination or 95% ethanol for molecular analysis. Worms were stained in Mayer's haematoxylin or acetocarmine, dehydrated in a graded ethanol series, cleared in methyl salicylate and mounted permanently in Canada balsam or Damar gum. Helminth specimens collected during the present study were deposited in collection of the Smithsonian National Museum of Natural History (NMNH), Washington, D.C. (Table 5). Specimens were examined using bright-field and Nomarski differential interference contrast (DIC) optics on an Olympus BX 51 microscope and illustrated using an attached

drawing tube. Measurements are given in micrometers (μm) unless otherwise specified and are expressed as those of the holotype followed by the minimum and maximum values of paratypes in parentheses. The length and width of vitelline follicles are expressed as averages and standard deviations of 10 random follicles distributed throughout the body. Characters expressed as a measurement followed by body length (BL) refer to the distance from the anterior end.

Genomic DNA was isolated from each species of *Plagioporus* (number of replicates [from separate individual worms] per species displayed in Table 5) using Qiagen DNAeasy Tissue Kit (Qiagen, Inc., Valencia, California, USA) following the instructions provided by the manufacturer. A region of the rRNA genome comprising the 3′ end of the 18S nuclear rRNA gene, internal transcribed spacer regions ITS1 and ITS2 (including 5.8S), and a partial sequence of the 28S rRNA gene (including variable domains D1–D3), were amplified from the extracted DNA by polymerase chain reaction (PCR) on a PTC-200 Peltier Thermal Cycler using forward primer ITSF (5′ CGC CCG TCG CTA CTA CCG ATT G-3′) and reverse primer 1500R (5′ GCT ATC CTG AGG GAA ACT TCG-3′). These PCR primers and multiple internal primers were used in sequencing reactions. The internal forward primers were digl2 (5′ AAG CAT ATC ACT AAG CGG-3′), 300F (5′ CAA GTA CCG TGA GGG AAA GTT G-3′) and 900F (5′ CCG TCT TGA AAC ACG GAC CAA G-3′) and the internal reverse primers were 300R (5′ CAA CTT TCC CTC ACG GTA CTT G-3′), digl2R (5′ CCG CTT AGT GAT ATG CTT-3′) and ECD2 (5′ CTT GGT CCG TGT TTC AAG ACG GG-3′) (for primers see Littlewood et al., 2000; Tkach et al., 1999, 2000, 2001, 2003; Tkach & Snyder, 2007). The resulting PCR products were excised from PCR gels using QIAquick Gel Extraction

Kit (Qiagen, Inc., Valencia, California, USA) following the manufacturer's instructions, cycle-sequenced using ABI BigDye™ chemistry (Applied Biosystems, Inc., Carlsbad, California, USA), ethanol-precipitated and run on an ABI 3130 Genetic Analyzer™. The sequences of the 3 new species herein described were assembled using Sequencher™ (GeneCodes Corp., Ann Arbor, Michigan, USA, Version 4.10.1) and deposited in GenBank (Table 5). The sequences were aligned using MAFFT version 6.611b (Katoh et al., 2005) with 1,000 cycles of iterative refinement and the genafpair algorithm. The boundaries between the 5.8S, ITS2 and 28S genes were located using the ITS2 Ribosomal Database (Keller et al., 2009). Pairwise sequence comparisons of the ITS1, 5.8S, ITS2 and 28S nuclear rRNA genes of the 2 new species of *Plagioporus* from this study and available sequences of *Plagioporus* from GenBank were calculated with MEGA v6 using the ‘‘compute pairwise differencesfunction,’’ with gaps treated using the ‘‘pairwise deletion’’ function (Tables 7 & 8). For phylogenetic analysis, sequences of opecoelids were obtained from GenBank (Table 6). The resulting alignment utilized 26 opecoelids, an acanthocolpid and used the brachycladiid *Zalophotrema hepaticum* Stunkard & Alvey, 1929 as the outgroup based on its phylogenetic position relative to the Opecoelidae (Olson et al., 2003). Phylogenetic analysis was performed using BI with MrBayes 3.2.6 software (Huelsenbeck & Ronquist, 2001) run on the CIPRES portal (Miller et al., 2010). The best nucleotide substitution model was estimated with jModeltest-2 (Darriba et al., 2012) as general time reversible with estimates of invariant sites and gamma-distributed among site-rate variation (GTR + I + Γ). The following model parameters were used in MrBayes: nst = 6, rates = invgamma, ngen = 5,000,000 and samplefreq = 1,000. Burn-in value was 4,000 estimated by plotting the log-

probabilities against generation and visualizing plateau in parameter values (sump burnin = 4,000), and nodal support was estimated by posterior probabilities (sumt) (Huelsenbeck et al., 2001) with all other settings left as default.

Description of *Plagioporus icatluri* n. sp.

Plagioporus ictaluri n. sp.

Type- and only known-host: Noturus lachneri Taylor, Ouachita Madtom (Siluriformes: Ictaluridae).

Type-locality: Middle Branch of Gulpha Creek off East Grand Avenue, Hot Springs, Garland County, U.S.A. (34° 30’ 33.17” N, 93 ° 00’ 32.48” W).

Site: Intestine.

Prevalence: 5 of 10 hosts (50%).

Intensity: 2-18 per host (mean 6).

Type-material: Holotype (USNM XXXXXXX), Paratype (USNM XXXXXXX-X).

Representative DNA sequences: Partial ITS1 and complete ITS2 regions, 5.8S gene, partial (D1–D3) 28S: GenBank accession no. NNXXXXXX, from 3 identical sequences.

Etymology: This species is named after the host family as it is the first species of *Plagioporus* to be described from an ictalurid.

Description (Fig. 3.1-4)

[Measurements based on 14 gravid wholemounts.] Body white to yellow in life, lanceolate with bluntly rounded ends, 818 (645-1242) long, 234 (176-321) wide.

Tegument smooth. Forebody slightly arched dorsally, 310 (229-376), representing 38 (30-40)% of body length (BL). Hindbody dorsally arched or not. Oral sucker subterminal to terminal, 85 (78-120) long by 95 (81-123) wide. Ventral sucker sunken, 145 (115-191) long by 151 (117-206) wide; width representing 65 (53-71)% of body width. Ratio of oral sucker to ventral sucker width 1.6 (1:1.4-1.7). Prepharynx 21 (15-26) long. Pharynx slightly separated from to slightly overlapping oral sucker, 48 (38-60) long, 49 (40-74) wide. Esophagus with or without turn, 107 (89-150) long. Intestinal bifurcation anterior to ventral sucker at 233 (186-332) BL, representing 28 (27-33)% of BL. Caeca extend posteriorly as far as the anterior 1/3 of the posterior testis. Postcaecal space 158 (97-286), representing 19 (15-24)% of BL.

Testis two, tandem, overlapping to contiguous; anterior testis 117 (85-184) long, 106 (93-187) wide, with anterior extent at 564 (423-803), representing 69 (59-77)% of BL; posterior testis longer than wide, overlapped by anterior testis by as much as 1/2 its length, 121 (104-236) long, 108 (81-202) wide, with anterior extent at 654 (495-956) BL, representing 80 (74-82)% of BL. Posttesticular space 40 (13-72), representing 5 (1.4-7.0)% of BL. Cirrus sac clavate, 200 (142-287) long, 60 (40-75) wide, length representing 25 (18-27)% of BL, overlapping the ventral sucker in the anterior 1/4-2/3. Internal seminal vesicle convoluted, S-shaped, 81 (56-117) long, 41 (29-70) wide, occupying 1/3- 1/2 of sac, connected to indistinct tubular region (likely *pars prostatica*) at 90º turn. Gonopore ventrolateral, sinistral, in forebody at 196 (158-292), representing 24 (23-29)% of BL.

Ovary ovoid, triangular, kidney bean-, tear drop-, to heart-shaped, submedian to median, overlaps anterior testis by 52 (0-82)% of ovary length, 86 (59-112) long, 84 (58-

109) wide, with anterior margin at 523 (411-718) BL, representing 64 (54-75)% of BL. Oviduct extends posterodorsally to posterolaterally from ovary, joins with canalicular seminal receptacle; seminal receptacle median, dorsal to anterior testis and caeca, extends posterior to ovary and overlaps anterior half of anterior testis. Laurer's canal extends anteriorly from seminal receptacle, with or without distal coil, opening sinistrally on dorsal surface anterior to ovary. Mehlis gland conspicuous. Uterus extends as far posteriorly to anterior 1/4 of anterior testis, with metraterm arising at level of posterior half of internal seminal vesicle, contains 9 (1-27) eggs. Eggs 71 (64-74) long, 40 (37-47) wide. Vitellarium follicular, in 2 lateral bunches, mostly ventral to caeca except for a few follicles at level of intestinal bifurcation and termination, anterior extent at 146 (129-197) BL, representing 18 (13-21)% of BL, extends as far anteriorly as midpoint of pharynx, posterior extent at 694 (474-982) BL, representing 85 (73-86)% of BL, extends as far posteriorly as anterior 1/4 of posterior testis. Average length of 10 follicles 35 (41 ± 11, 28-63), average width of 10 follicles 29 (36 ± 11, 22-57). Vitelline reservoir median, dorsal to ovary and anterior testis, ventral to seminal receptacle.

Excretory vesicle sac-like, with maximum anterior extent overlapping posterior 1/2 of anterior testis immediately posterior to ovary, 171 (103–291) long, representing 21 (15-27)% of BL; pore terminal.

Remarks

Plagioporus ictaluri n. sp. belongs in *Plagioporus* because of its possesses all of the diagnostic features of the genus (Fayton & Andres, 2016; Cribb, 2005) with one notable exception, in having an excretory bladder that reaches anteriorly to the anterior testis instead of being restricted to the post-testicular area.

Plagioporus ictaluri n. sp. is easily distinguished from its North American congeners in the distribution and position of the vitellarium. The new species can be distinguished from *P. serotinus* Stafford, 1904, *P. cooperi* (Hunter & Bangham, 1932) Price, 1934, *P. lepomis* Dobrovolny, 1939, *P. boleosomi* (Pearse, 1924) Peters, 1957, *P. hypentelii* Hendrix, 1973, *P. macrouterinus* Haderlie, 1953, *P. kolipinskii* Tracey, Choudhury, Cheng, & Ghosh, 2009, *P. siliculus* Sinitsin, 1931, *P. shawi* (McIntosh, 1939) Margolis, 1970 and *P. hageli* Fayton & Andres, 2016 in the absence of confluent vitelline fields in the posttesticular space. *Plagioporus ictaluri* n. sp. can be distinguished from *P. sinitsini* Mueller, 1934, *P. serratus* Miller, 1940 and *P. chiliticorum* (Barger & Esch, 1999) Cribb 2005 in possession of vitellarium extending posteriorly only to the anterior quarter of the anterior testis as opposed to one extending to the posterior margin of the posterior testis or beyond. *P. loboides* (Curran, Overstreet, & Tkach, 2007) can be distinguished from the new species in lacking vitellaria in the forebody. *Plagioporus ictaluri* n. sp. is morphologically most similar to *P. sinitsini*, *P. serratus*, *P. chiliticorum* and *P. hypentelii* but can be distinguished from these species in having an excretory vesicle overlapping the anterior testis versus one confined to the posttesticular space (*P. sinitsini*, *P. serratus* and *P. chiliticorum*) or reaching only to the posterior testis (*P. hypentelii*). The new species can be further distinguished from *P. sinitsini* and *P. serratus* in parasitizing the intestine as opposed to the gall bladder of its host; from *P. hypentelii* in having a longer esophagus (89-150 μm as opposed to 42-90 μm) and caeca extending only to the anterior quarter of the posterior testis as opposed to the posterior end; and from *P. chilitcorum* in lacking a bipartite seminal vesicle and testis well separated from the posterior end.

Plagioporus ictaluri n. sp. is the first species of *Plagioporus* to be described from ictalurids. It was not found in the following hosts also examined from the type locality: *Etheostoma radiosum* (Hubbs & Black) (n=10, 25 November 2014, n=15, 23 May 2015), *Campostoma spadiceum* (Girard) (n=5, 23 May 2015), *Lepomis cyanellus* Rafinesque (n=1, 26 October 2013) and *Fundulus catenatus* (Storer) (n=12, 25 November 2014, n=2, 26 October 2014). It was also not found in other species of madtoms (*Noturus eleutherus* Jordan, *Noturus nocturnus* Jordan & Gilbert, *Noturus taylori* Douglas) in the Ouachita River drainage, nor in *Ictalurus* spp. from the Ouachita River drainage and ictalurids from adjacent drainages in Arkansas and Oklahoma (Table 9).

Description of *Plagioporus carolini* n. sp.

Plagioporus carolini n. sp.

Type- and only known-host: Cottus carolinae (Gill), Banded Sculpin (Scorpaeniformes: Cottidae)

Type-locality: Flint Creek off Fairmont Road at Springtown, Benton County, Arkansas (36º 15’ 9.9” N, 94º 26’ 25.8” W).

Site: Intestine.

Prevalence: 8 of 20 hosts (40%).

Intensity: 1-15 per host (mean 6).

Type-material: Holotype (USNM XXXXXXX), Paratype (USNM XXXXXXX-X).

Representative DNA sequences: includes the partial ITS1 and complete ITS2 regions, 5.8S gene, and partial 28S (D1–D3 regions): GenBank accession no. NNXXXXXX, from 3 identical sequences.

Etymology: This species is named after the host species as it is the first of its genus to be described from a cottid east of the Rocky Mountains.

Description (Fig. 4.5-8)

[Measurements based on 10 gravid wholemounts.] Body white to yellow in life, nearly cylindrical with bluntly rounded ends, 626 (512-784) long, 182 (153-238) wide. Tegument smooth. Forebody with slight dorsal arch, 237 (71-270) long, representing 38 (29-38)% of body length (BL). Hindbody with or without dorsal arch in posterior half. Oral sucker subterminal to terminal, 78 (70-103) long by 101 (80-116) wide. Ventral sucker sunken, 128 (119-174) long by 154 (127-203) wide; width representing 85 (80-92)% of body width. Ratio of oral sucker to ventral sucker width 1:1.6 (1:1.6-1.9). Prepharynx 16 (15-40) long. Pharynx continguous to overlapping oral sucker by as much as half of its length, 53 (35-60) long, 58 (44-66) wide. Esophagus with turn or turns, 73 (36-73) long. Intestinal bifurcation anterior to ventral sucker at 177 (55-209) BL, representing 28 (24-33)% of BL. Caeca extend posteriorly as far as the anterior 1/3 of the posterior testis. Postcaecal space 180 (100-180), representing 28 (15-29)% of BL.

Testis two, oblique, overlapped to contiguous; anterior testis 129 (105-149) long, 117 (92-139) wide, with anterior extent at 428 (418-516), representing 68 (62-71)% of BL; posterior testis overlaps anterior testis by as much as 2/3 of its length, 131 (110-150) long, 134 (102-157) wide, with anterior extent at 492 (374-616) BL, representing 79 (71-79)% of BL. Posttesticular space 6 (6-39), representing 1 (1.0-6.3)% of BL. Cirrus sac clavate, overlaps the ventral sucker in anterior 1/4-2/3 of length, 144 (126-188) long, 56

(34-56) wide, length representing 23 (19-27)% of BL. Internal seminal vesicle convoluted, S-shaped, 63 (42-84) long, 25 (20-45) wide, occupies 1/3 to 1/2 of sac, communicates with indistinct tubular region (likely pars protatica and ejaculatory duct) at 90º turn. Gonopore ventrolateral, sinistral, in forebody at 154 (125-203), representing 25 (23-27)% of BL.

Ovary ovoid, triangular, kidney bean-, to tear drop-shaped, dextral to anterior testis, overlaps anterior testis ventrally by 99 (71-100)% of its length and as much as 2/3 of its width, 85 (57-85) long, 93 (42-100) wide, with anterior margin at 427 (313-536) BL, representing 68 (61-73)% BL. Oviduct extends anterodorsally from ovary, joins with canalicular seminal receptacle; seminal receptacle median, dorsal to anterior testis and ovary, extends posteriorly as far as level of posterior testis. Laurer's canal extends anteriorly from seminal receptacle, opens sinistrally on dorsal surface anterior to ovary. Mehlis gland conspicuous. Uterus extends as far posteriorly to anterior 1/4 of anterior testis, with metraterm arising at level of posterior half of cirrus sac, contains 6 (1-13) eggs. Eggs 77 (68-78) long, 41 (36-46) wide. Vitellarium follicular, in 2 lateral bunches, mostly ventral to caeca except for a few follicles at level of intestinal bifurcation and termination, anterior extent at 121 (86-121) BL, representing 19 (15- 21)% of BL, extends as far anteriorly as midpoint of pharynx, posterior extent at 502 (413-662) BL, representing 80 (74-87)% of BL, extends as far posteriorly as anterior 1/3 of posterior testis. Average length of 10 follicles 38 (35 ± 7, 25-36), average width of 10 follicles 30 (30 ± 4, 23-37). Vitelline reservoir median, dorsal to ovary and anterior testis, ventral to seminal receptacle.

Excretory vesicle sac-like, with maximum anterior extent overlapping posterior 1/2 of anterior testis and reaching level of ovary, 126 (116–171) long, representing 20 (18-28)% of BL; pore terminal.

Remarks

Plagioporus carolini n. sp. belongs in *Plagioporus* because of its possesses all of the diagnostic features of the genus (Fayton & Andres, 2016; Cribb, 2005) with one notable exception, in having an excretory bladder that reaches anteriorly to the anterior testis instead of being restricted to the post-testicular area.

Plagioporus carolini n. sp. is easily distinguished from its North American congeners in the extent of the testicular space and the relative position of the excretory vesicle and ovary. It can be distinguished from *P. serotinus*, *P. cooperi*, *P. lepomis*, *P. boleosomi*, *P. macrouterinus*, *P. kolipinskii*, *P. siliculus*, *P. shawi*, *P. hageli*, *P. sinitsini*, *P. serratus*, *P. chiliticorum* and *P. loboides* in having a testicular space occupying more than 50% of the length of the hindbody (61-91% of hindbody length in *Plagioporus carolini* n. sp.). *Plagioporus carolini* n. sp. can be distinguished from the remaining congeners, *P. hypentelii* and *P. ictaluri*, in having an excretory vesicle extending as far anteriorly as the level of the ovary. In *P. hypentelii* the excretory vesicle only reaches the posterior testis. While the excretory vesicle of *P. ictaluri* overlaps the anterior testis, it does not reach the level of the ovary. The new species is similar to *P. sinitsini*, *P. serratus* and *P. chiliticorum* in having the vitellarium restricted in 2 lateral bands without a confluent vitelline field in the posttesticular space. It is further similar to *P. sinitsini* and *P. serratus* in possessing a short posttesticular space. It is also similar to *P. hypentelii* in testis size and vitellarium distribution; although *P. hypentelii* has a confluent vitelline

field in the posttesticular space, it is only vaguely confluent, with lateral fields connected posterior to the testis by a follicle or two. The new species is morphologically most similar to *P. ictaluri* in having large testes, a short postesticular space, vitellarium in 2 lateral bands largely ventral to the caeca in the hindbody and extending approximately only to the level of the anterior testis, caeca well separated from the posterior end, a convoluted, s-shaped internal seminal vesicle and an excretory vesicle overlapping the anterior testis. *Plagioporus carolini* n. sp. can be distinguished from *P. ictaluri* in having oblique versus tandem testis, ovary dextral as opposed to one that is submedian to median, ovary overlaps the anterior testis by 71-100 (average 91)% of its length as opposed to 0-82 (average 46)%, ventral sucker occupying 80-92% of the body width as opposed to 53-71% , oral sucker occupying 49-58% of the body width compared to 36-47% and pharynx occupying 28-36% of the body width as opposed to 21-26%. The testes of *Plagioporus carolini* n. sp. also tend to be larger; the anterior testis occupies 17-28% BL versus 11-18% BL. The testicular space also tends to comprise a greater portion of the hindbody in the new species, occupying 61-91% of the hindbody as opposed to 47-69% in *P. ictaluri*. In addition, the esophagus of the new species tends to be shorter, representing 7-12% BL as opposed to 11-14% in *P. ictaluri*.

Plagioporus carolini n. sp. is the second species of *Plagioporus* to be described from a cottid and is the only species from a cottid currently retained in the genus. It was not found in the following hosts also examined from the type locality (numbers of individuals and dates of capture in parentheses): *Etheostoma squamosum* (Agassiz) (n=14, 15 May 2015; n=6, 21 May 2015), *Campostoma anomalum* Rafinesque (n=16, 15 May 2015), *Notropis boops* Gilbert (n=7, 13 June 2014), *Chrosomus erythrogaster*

Rafinesque (n=5, 15 May 2015; n=1, 21 May 2015), *Luxilus cardinalis* (Mayden) (n=4,14 May 2014; n=11, 21 May 2015; n=2, 13 June 2014), *Notropis nubilis* (Forbes) (n=1, 21 May 2015), *Semotilus atromaculatus* (Mitchill) (n=16, 16 May 2015; n=8, 13 June 2014) and *Ambloplites rupestris* (Rafinesque) (n=1, 13 June 2014). A morphologically similar form was found in the intestine of *N. exilis* at the type locality at Flint Creek. This form was previously reported from Flint Creek by McAllister et al., (2015). Measurements of 3 mature specimens fall within the range of *Plagioporus carolini* n. sp. These specimens have an excretory vesicle extending to the level of the ovary, oblique testis and an ovary that is dextral to and parallel to the anterior testis. Thus, the new species may also parasitize *N. exilis*; we await sequence data to test this hypothesis of co-occurrence. Forms consistent with *Plagioporus carolini* n. sp. from ictalurid hosts were only found in Flint Creek and not elsewhere in the Arkansas River drainage nor in other drainages in Arkansas and Oklahoma (Table 9). The new species was not found in *C. carolinae* sampled elsewhere in the Arkansas River drainage, *Cottus* spp. from the White River drainage in Arkansas, *Cottus* spp. from the Osage River drainage to the North in southern Missouri nor in *C. carolinae* from the Cumberland River drainage in Tennessee (Table 10). In Arkansas, cottids are restricted to the Ozark plateau and are thus not found in the Ouachita drainage from which *Plagioporus ictaluri* n. sp. was described (Robison & Buchanan, 1988).

Molecular Analysis

Sequence lengths of the partial ITS1 rDNA gene used for pairwise comparisons for *P. boleosomi*, *P. chiliticorum*, *P. hageli*, *P. kolipinskii*, *P. sinitsini*, *P. shawi*, *Plagioporus ictaluri* n. sp. and *Plagioporus carolini* n. sp. were 613, 615, 811, 661, 600,

451, 900 and 867 respectively. The length of the complete 5.8SrDNA gene for all of these species was 156 bp and lengths of the partial 28S rDNA gene ranged from 1,196-1,199 bp. The length of the complete ITS2 rDNA gene was 250 bp for all species of *Plagioporus* examined except for those of *P. shawi* and *P. kolipinskii*, which had respective lengths of 240 bp and 251 bp. No intraspecific variation was observed in sequences of *P. ictaluri* n. sp. or *P. carolini* n. sp.

Pairwise comparison of sequences all species of *Plagioporus* revealed that *Plagioporus ictaluri* n. sp. and *Plagioporus carolini* n. sp. were most similar to one another, with similarities of 99.2% and 99.8%, in the partial ITS1 (Table 7) and partial 28S rDNA genes, respectively, and in turn both species were most similar to *P. sinitsini* and *P. chiliticorum*. *Plagioporus ictaluri* n. sp. was 86.3% and 96.9% similar to *P. sinitsini* and 85.7% and 97.2% similar to *P. chiliticorum* in the partial ITS1 and partial 28S rDNA genes, respectively, whereas *Plagioporus carolini* n. sp. was 86.4 % and 97.1% similar to *P. sinitsini* and 85.2% and 97.4% similar to *P. chiliticorum* in the partial ITS1 and partial 28S rDNA genes, respectively. In the complete ITS2 (Table 6) rDNA gene, the 2 new species were most similar to one another with a similarity of 99.6%, and in turn both were most similar to *P. sinitsini*, *P. hageli* and *P. chiliticorum*. *Plagioporus ictaluri* n. sp. and *Plagioporus carolini* n. sp. were respectively 95.6%, 95.6% and 94.8% and 96.0%, 96.0% and 95.2% similar to *P. sinitsini*, *P. hageli* and *P. chiliticorum*, respectively, in the complete ITS2 rDNA gene. While species of *Plagioporus* diverged minimally from one another in the complete 5.8S (Table 7) rDNA gene, with a maximum divergence of 3 base pairs, the 2 new species were most similar to one another with a similarity of 100%.

The alignment of the partial 28SrDNA sequences of the 2 new species and related species from GenBank resulted in a dataset with 1,229 characters, with 784 conserved sites, 442 variable sites and 306 parsimony informative sites. My BI analysis (Figure 5) resolved *Plagioporus ictaluri* n. sp. and *Plagioporus carolini* n. sp. as sister taxa with high support and they were in-turn sister to *P. chilitcorum* and in-turn sister to *P. sinitsini* also with high support. The clade formed by these 4 eastern Nearctic species was sister to one containing *P. hageli*, *P. loboides* and *P. kolipinskii* with low support. *Plagioporus boleosomi* was resolved as sister to these 7 species of *Plagioporus* with high support and *P. shawi* was resolved as sister to all other species of *Plagioporus* with low support. Consistent with Fayton & Andres (2016), *Plagioporus* was resolved as sister to freshwater plagioporines from the Palearctic (*Neoplagioporus* Shimazu, 1990 and *Urorchis* Ozaki, 1927).

Discussion

Morphologically, *Plagioporus ictaluri* n. sp. and *Plagioporus carolini* n. sp. are most similar to each other and in turn were similar to congeners parasitizing cyprinids (*P. chiliticorum* and *P. sinitsini*), catostomids (*P. sinitsini* and *P. hypentelii*), or hiodontids (*P. serratus*) in the eastern Nearctic. Pairwise comparisons of partial ITS1, complete 5.8S, complete ITS2 and partial 28S rDNA genes along with my BI analysis confirmed a close similarity between the 2 new species. My BI analysis and pairwise comparisons of partial ITS1 and partial 28S rDNA genes also corroborate a close similiarity of the 2 new species to *P. chiliticorum* and *P. sinitsini.* While *Plagioporus ictaluri* n. sp. and *Plagioporus carolini* n. sp. were most similar to *P. sinitsini* and *P. hageli* followed by *P. chiliticorum* in the complete ITS2 rDNA gene, I suspect that this similarity of the 2 new

species to *P. hageli* is an artifact of the low number of informative sites at this locus. My BI analysis resolved the relationship between *P. sinitsini*, *P. chiliticorum*, *Plagioporus carolini* n. sp. and *Plagioporus ictaluri* n. sp. with high support, and these 4 species are notably the only members of *Plagioporus* without a uterus extending to the posterior end that lack a confluent vitelline field in the posttesticular space (excluding *P. serratus*, for which sequence data are not available). The morphological similarity between the 2 new species, their low degree of divergence in the ITS region and 28S rDNA gene, and their apparent endemicity to drainages that are adjacent to one another (Tables 9 and 10) together supports the notion that these species are sister to one another and may indicate a host switching event between cottids and ictalurids. The morphological and genetic affinity of the 2 new species to *P. chilitcorum* and *P. sinitsini* may indicate a host switching event between these hosts and cyprinids or possibly catostomids in the eastern Nearctic. Inclusion of additional species of *Plagioporus* in subsequent phylogenies may clarify the relationships between congeners and elucidate the directionality of these possible host-switching events.

My BI analysis is consistent with that of Fayton & Andres (2016) except for the placement of *P. boleosomi*, which was resolved as sister to all other species of *Plagioporus* excluding *P. shawi* with high support. Fayton & Andres resolved *P. boleosomi* as sister to *P. kolipinskii*, *P. hageli* and *P. loboides* with moderate support. I suspect that the placement of *P. bolesomi* in my BI analysis reflects its true relationship with its congeners and will test this hypothesis with the addition of other forms from percids in subsequent phylogenies.

Gibson and Bray (1982) proposed that *Plagioporus* be restricted to 'freshwater forms with a short excretory vesicle,' reaching anteriorly at most to the level of the posterior testis, thereby separating it from marine genera with which it was often confused, and Cribb's (2005) placement of species in *Plagioporus* relies heavily on the validity of this short excretory bladder as a diagnostic trait. While 12 of the 16 valid species of *Plagioporus* in the Nearctic have excretory bladders that are either pretesticular or at the level of the posterior testis, *P. siliculus*, *Plagioporus ictaluri* n. sp., *P. shawi* and *Plagioporus carolini* n. sp. have excretory vesicles that extend at least to the level of the anterior testis and to the level of the ovary in the latter 2 species. In the BI analysis of Fayton & Andres (2016), as *P. shawi* was resolved as sister to its congeners with low support, these authors refrained from making any taxonomic changes to accommodate the long excretory vesicle of this species. Given that *Plagioporus carolini* n. sp. and *Plagioporus ictaluri* n. sp. are clearly nested within *Plagioporus* in my BI analysis and the condition of their excretory vesicles, the following amendment to *Plagioporus* is proposed: excretory vesicle of variable length, may reach ovary. The possession of such an excretory vesicle is also seen in some freshwater plagioporine genera from Japan that were resolved as sister to *Plagioporus* in my BI analysis. The excretory vesicles of *Urorchis goro* Ozaki, 1927, *Urorchis acheilognathi* Yamaguti, 1934, *Neoplagioporus zacconis* (Yamaguti, 1934) Shimazu, 1990 and *Neoplagioporus elongatus* (Goto et Ozaki, 1930) Shimazu, 1990 extend anteriorly to the level of the anterior testis and in *Urorchis imba* reaches the level of the ovary (Shimazu 1990a, 1990b). Thus, the length of the excretory vesicle may not necessarily be useful in distinguishing freshwater plagioporine genera from marine genera with which they are

often confused, particularly in forms where the ovary can be parallel to the anterior testis as in *Plagioporus carolini* n. sp. This study includes the first species of *Plagioporus* to be described from an ictalurid host, the first species in the genus to be described from a cottid east of the Rocky Mountains, and also the first species in the genus to be described from Arkansas.

Table 5 New species of *Plagioporus* collected from the Nearctic and their respective hosts, collection localities, GenBank accession number (with number of replicates in parenthesis) and deposition information.

Species	**Host**	**Collection Locality and Date**		**GenBank**	**NMNH**
Plagioporus carolini n. sp.	*Cottus carolinae* (Gill)	Flint Creek, A.R.	03/19/14	NNXXXXXX (3)	XXXXX
Plagioporus ictaluri n. sp.	*Noturus lachneri* Taylor	Gulpha Creek, A.R.	11/26/14	NNXXXXXX (3)	XXXXX

Table 6 Sequences obtained from GenBank used for phylogenetic analysis

Family	Species	Host	GenBank No.	Reference
Brachycladiidae	*Zalophotrema hepaticum* Stunkard & Alvey, 1929	*Zalophus californianus* (Lesson)	AY222255	Olson et al. (2003)
Acanthocolpidae	*Stephanostomum pristis* (Deslongchamps, 1824)	*Phycis phycis* (Linnaeus)	DQ248222	Bray et al. (2005)
Opecoelidae	*Allopodocotyle margolisi* Gibson, 1995	*Coryphaenoides mediterraneus* (Giglioli)	KU320596	Bray et al. (2016)
Opecoelidae	*Anomalotrema koiae* Gibson & Bray, 1984	*Sebastes viviparus* Krøyer	KU320595	Bray et al. (2016)
Opecoelidae	*Bathycreadium brayi* Pérez-del-Olmo, Dallarés, Carrassón & Kostadinova, 2014	*Trachyrincus scabrus* (Rafinesque)	JN085948	Constenla et al. (2011)
Opecoelidae	*Buticulotrema thermichthysi* Bray, Waeschenbach, Dyal, Littlewood & Morand , 2014	*Thermichthys hollisi* (Cohen, Rosenblatt & Moser)	KF733984	Bray et al. (2014)
Opecoelidae	*Dimerosaccus oncorhynchi* (Eguchi, 1931)	*Oncorhynchus masou* (Brevoort)	FR870252	Shedko et al. (2015)
Opecoelidae	*Gaevskajatrema halosauropsi* Bray & Campbell, 1996	*Halosauropsis macrochir* (Günther)	AY222207	Olson et al. (2003)
Opecoelidae	*Macvicaria mormyri* (Stossish, 1885)	Unidentified fish host	AF184256	Tkach et al. (2001)
Opecoelidae	*Macvicaria obovata* (Molin, 1859)	*Cyclope neritea* (Linnaeus)	JQ694147	Born-Torrijos et al. (2012)
Opecoelidae	*Neolebouria lanceolata* Andres, Pulis & Overstreet, 2014	*Polymixia lowei* (Günther)	KJ001210	Andres et al. (2014)
Opecoelidae	*Neoplagioporus ayu* (Takahashi, 1928)	*Plecoglossus altivelis altivelis* (Temminck & Schlegel)	KX553947	Fayton et al. (2016)
Opecoelidae	*Neoplagioporus elongatus* (Goto & Ozaki, 1930)	*Sarcocheilichthys variegatus microoculus* Mori	KX553948	Fayton et al. (2016)
Opecoelidae	*Neoplagioporus zacconis* (Yamaguti, 1934)	*Opsariichthys platypus* (Temminch & Schlegel)	KX553949	Fayton et al. (2016)

Opecoelidae	*Opecoeloides fimbriatus* (Linton, 1910)	*Micropogonias undulatus* (Linnaeus)	KJ001211	Andres et al. (2014)
Opecoelidae	*Plagiocirrus loboides* Curran, Overstreet & Tkach, 2007	*Fundulus nottii* (Agassiz)	EF523477	Curran et al. (2007)
Opecoelidae	*Plagioporus boleosomi* (Pearse, 1924)	*Percina maculata* (Girard)	KX553953	Fayton et al. (2016)
Opecoelidae	*P. chiliticorum* (Barger & Esch, 1999)	*Notropis chiliticus* (Cope)	KX553943	Fayton et al. (2016)
Opecoelidae	*P. hageli* Fayton & Andres, 2016	*Oncorhynchus mykiss* (Walbaum)	KX553950	Fayton et al. (2016)
Opecoelidae	*P. kolipinskii* Tracey, Choudhury, Cheng & & Ghosh, 2009	*Gasterosteus aculeatus* Linnaeus	KX553952	Fayton et al. (2016)
Opecoelidae	*P. shawi* (McIntosh, 1939)	*Oncorhynchus tshawytscha*	KX553951	Fayton et al. (2016)
Opecoelidae	*P. sinitsini* Mueller, 1934	*Notemigonus crysoleucas* (Mitchill)	KX553944	Fayton et al. (2016)
Opecoelidae	*Podocotyloides brevis* Andres & Overstreet, 2013	*Conger esculentus* Poey	KJ001212	Andres et al. (2014)
Opecoelidae	*Pseudopecoeloides tenuis* Yamaguti, 1940	*Priacanthus hamrur* (Forsskål)	KU320605	Bray et al. (2016)
Opecoelidae	*Urorchis acheiloghathi* Yamaguti, 1934	*Tanakia limbata* (Temminck &	KX553945	Fayton et al. (2016)
Opecoelidae	*Urorchis goro* Ozaki, 1927	*Rhinogobius* sp.	KX553946	Fayton et al. (2016)

Table 7 Pairwise comparisons of percent nucleotide similarity and the number of base pair differences (in parentheses) for the 28S, ITS-2 and 5.8S of species of *Plagioporus.*

		Plagioporus ictaluri n. sp.	*Plagioporus boleosomi*	*Plagioporus chilliticorum*	*Plagioporus hageli*	*Plagioporus kolipinskii*	*Plagioporus sinitsini*	*Plagioporus shawi*	*Plagioporus loboides*
28S	*Plagioporus carolini* n. sp.	99.8 (3)	96.7 (43)	97.4 (34)	97.0 (39)	96.1 (52)	97.1 (38)	95.1 (64)	96.9 (38)
28S	*Plagioporus ictaluri* n. sp.	-	96.5 (46)	97.2 (37)	96.8 (42)	96.0 (53)	96.9 (41)	94.9 (67)	96.7 (40)
ITS-2	*Plagioporus carolini* n. sp.	99.6 (1)	94.8 (13)	95.2 (12)	96.0 (10)	94.4 (14)	96.0 (10)	86.3 (33)	NA
ITS-2	*Plagioporus ictaluri* n. sp.	-	94.4 (14)	94.8 (13)	95.6 (11)	94.0 (15)	95.6 (11)	85.8 (34)	NA
5.8S	*Plagioporus carolini* n. sp.	100 (0)	98.7 (2)	98.7 (2)	98.1 (3)	98.7 (2)	98.1 (3)	98.7 (2)	NA
5.8S	*Plagioporus ictaluri* n. sp.	-	98.7 (2)	98.7 (2)	98.1 (3)	98.7 (2)	98.1 (3)	98.7 (2)	NA

Table 8 Pairwise comparisons of percent nucleotide similarity and the number of base pair differences (in parentheses) for the ITS-1 (above the diagonal) of species of *Plagioporus.*

	Length of ITS-1 (bp)	*Plagioporus ictaluri* n. sp.	*Plagioporus boleosomi*	*Plagioporus chiliticorum*	*Plagioporus hageli*	*Plagioporus kolipinskii*	*Plagioporus sinitsini*	*Plagioporus shawi*
Plagioporus carolini n. sp.	944	99.2 (7)	84.0 (105)	85.2 (97)	82.3 (116)	77.0 (153)	86.4 (86)	82.3 (85)
Plagioporus ictaluri n. sp.	906	-	84.0 (104)	85.7 (93)	82.7 (112)	76.6 (154)	86.3 (86)	82.7 (82)
Plagioporus boleosomi	670	-	-	85.4 (96)	83.8 (107)	76.8 (152)	88.7 (72)	82.9 (83)
Plagioporus chiliticorum	664	-	-	-	84.4 (102)	77.1 (148)	87.6 (79)	82.2 (86)
Plagioporus hageli	865	-	-	-	-	77.3 (155)	85.0 (95)	82.3 (86)
Plagioporus kolipinskii	706	-	-	-	-	-	79.3 (130)	82.0 (85)
Plagioporus sinitsini	647	-	-	-	-	-	-	83.4 (79)
Plagioporus shawi	527	-	-	-	-	-	-	-

Table 9 Ictalurids negative for *Plagioporus carolini* n. sp. and *Plagioporus ictaluri* n. sp.

Host	Number	Site of Collection	Date
Noturus eleutherus Jordan	7	Little River, McCurtain Co., Red River drainage, OK	10/10/2015
Noturus eleutherus Jordan	2	Little Missouri River, Clark Co., Ouachita River drainage, AR	24/11/2014
Noturus nocturnus Jordan & Gilbert	1	Little River, Little River Co., Red River drainage, AR	10/25/2013
Noturus nocturnus Jordan & Gilbert	3	Little River, McCurtain Co., Red River drainage, OK	06/17/2013
Noturus nocturnus Jordan & Gilbert	6	Ouachita River, Montgomery Co., AR	10/13/2015
Noturus albater Taylor	1	Madison Co., White River, AR	15/10/2015
Noturus albater Taylor	6	Crooked Creek, Marion Co., White River drainage, AR	06/25/2013
Noturus maydeni Egge	2	Town Creek, Fulton County, White River drainage, AR	06/25/2013
Noturus maydeni Egge	20	Spring River, Fulton County, White River drainage, AR	07/08/2015
Noturus gyrinius (Mitchill)	7	Rolling Fork River, Sevier Co., Red River drainage,	10/24/2013
Noturus taylori Douglas	16	Caddo River, Montgomery Co., Ouachita River drainage, AR	12/18/2013
Noturus exilis Nelson	8	La Fave River, Perry Co., Arkansas River drainage, AR	10/14/2015
Noturus exilis Nelson	30	Crooked Creek, Marion Co., White River drainage, AR	07/26/2013
Noturus exilis Nelson	9	Spirit Creek, Franklin Co., White River drainage, AR	07/24/2014
Noturus exilis Nelson	5	Spirit Creek, Franklin Co., White River drainage, AR	07/05/2015
Noturus exilis Nelson	1	Mill Creek, Johnson Co., White River drainage, AR	06/07/2015
Noturus exilis Nelson	2	Little Minnow Creek, Johnson Co., White River drainage, AR	06/07/2015
Noturus exilis Nelson	3	Washita Creek, Johnson Co., White River drainage, AR	06/07/2015
Noturus exilis Nelson	2	N. Fork White Oak Creek, Franklin Co., White River drainage, AR	07/05/2015
Noturus exilis Nelson	5	Fane Creek, Franklin Co., White River drainage, AR	07/05/2015
Noturus exilis Nelson	7	Illinois River tributary, Cherokee Co., Arkansas River drainage, OK	06/05/2014
Ameiurus natalis (Leseur)	20	Cane Creek Lake, Lincoln Co., Ouachita River drainage, AR	06/27/2014
Ictalurus furcatus (Valenciennes)	1	Cane Creek Lake, Lincoln Co., Ouachita River drainage, AR	06/27/2014
Ictalurus punctatus (Rafinesque)	5	Crooked Creek, Marion Co., White River drainage, AR	07/23/2014
Ameriurus melas (Rafinesque)	3	Black Fox Hollow Creek, Adair Co., Illinois River drainage, OK	06/05/2015

Table 10 *Cottus* spp. negative for *Plagioporus carolini* n. sp.

Host	Number	Site of Collection	Date
Cottus carolinae (Gill)	3	Marion Co., White River drainage, AR	02/17/2013
Cottus carolinae (Gill)	4	Spavinaw Creek, Benton Co., Arkansas River drainage, AR	06/17/2013
Cottus carolinae (Gill)	3	Water Creek, Searcy Co., White River drainage, AR	06/14/2013
Cottus carolinae (Gill)	3	Poke Creek, Independence Co., White River drainage, AR	06/25/2013
Cottus carolinae (Gill)	3	Calico Creek, Izard Co., White River drainage, AR	06/25/2013
Cottus carolinae (Gill)	10	Calico Creek, Izard Co., White River drainage, AR	07/07/2015
Cottus carolinae (Gill)	15	Bennett Spring, Dallas Co., Osage River drainage, MO	12/18/2013
Cottus bairdii Girard	10	Bennett Spring, Dallas Co., Osage River drainage, MO	12/18/2013
Cottus immaculatus (Kinziger & Wood)	20	Spring River, Fulton Co., White River drainage, AR	07/27/2013
Cottus carolinae (Gill)	20	Bledsoe Creek, Sumner Co., Cumberland River drainage, TN	05/20/2014

Figure 3. Plagioporus ictaluri n. sp. from the intestine of *Noturus lachneri*. 1, Ventral view; 2, Dorsal view; 3, Terminal genitalia; 4, Lateral view of female complex. Scale bars for 1-2: 100 µm, Scale bars for 3-4: 50 µm

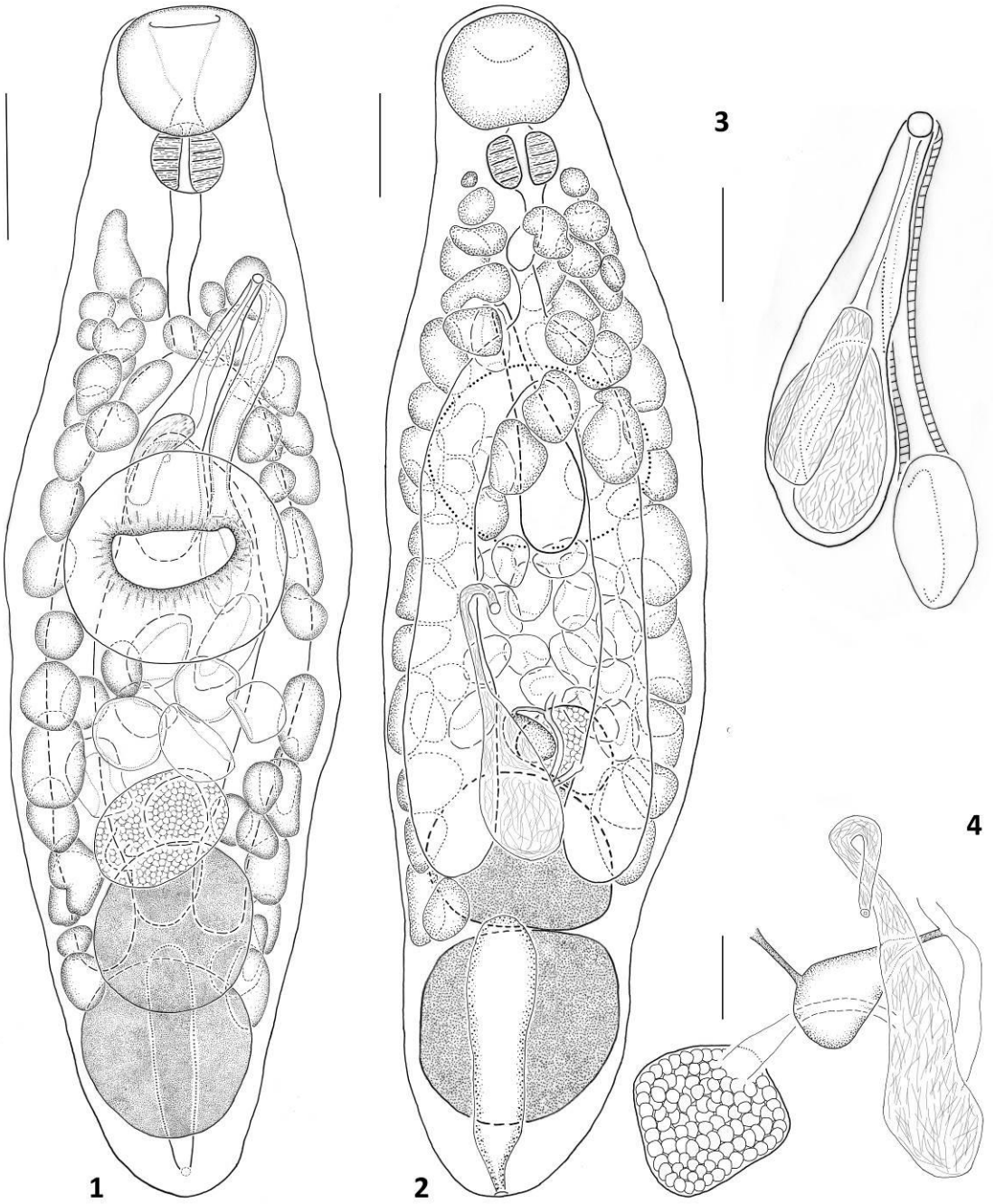


Figure 4. Plagioporus carolini n. sp. from the intestine of *Cottus carolinae*. 5, Ventral view; 6, Dorsal view; 7, Terminal genitalia; 8, Dorsal view of female complex. Scale bars for 5-6: 100 µm, Scale bars for 7-8: 50 µm

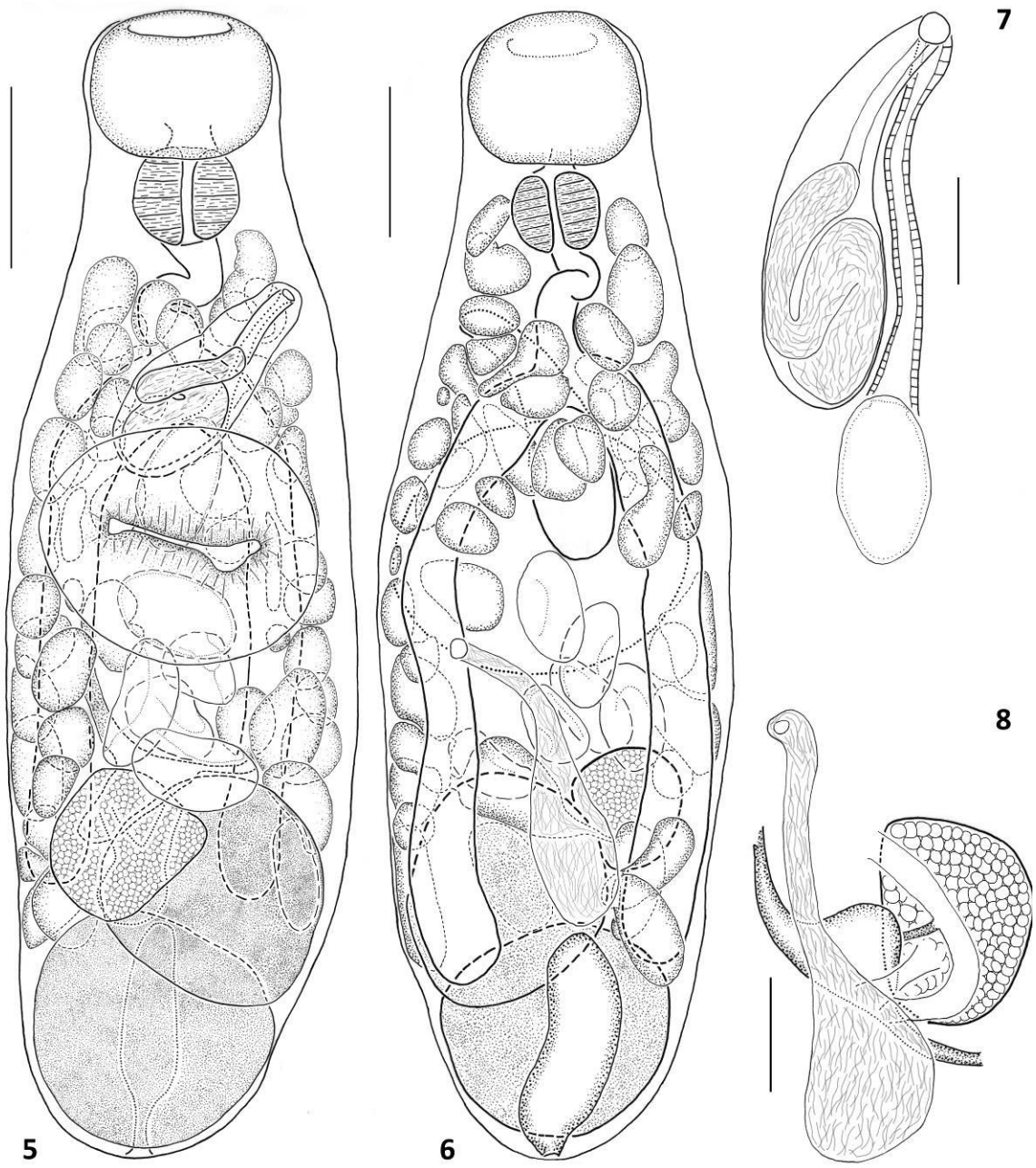
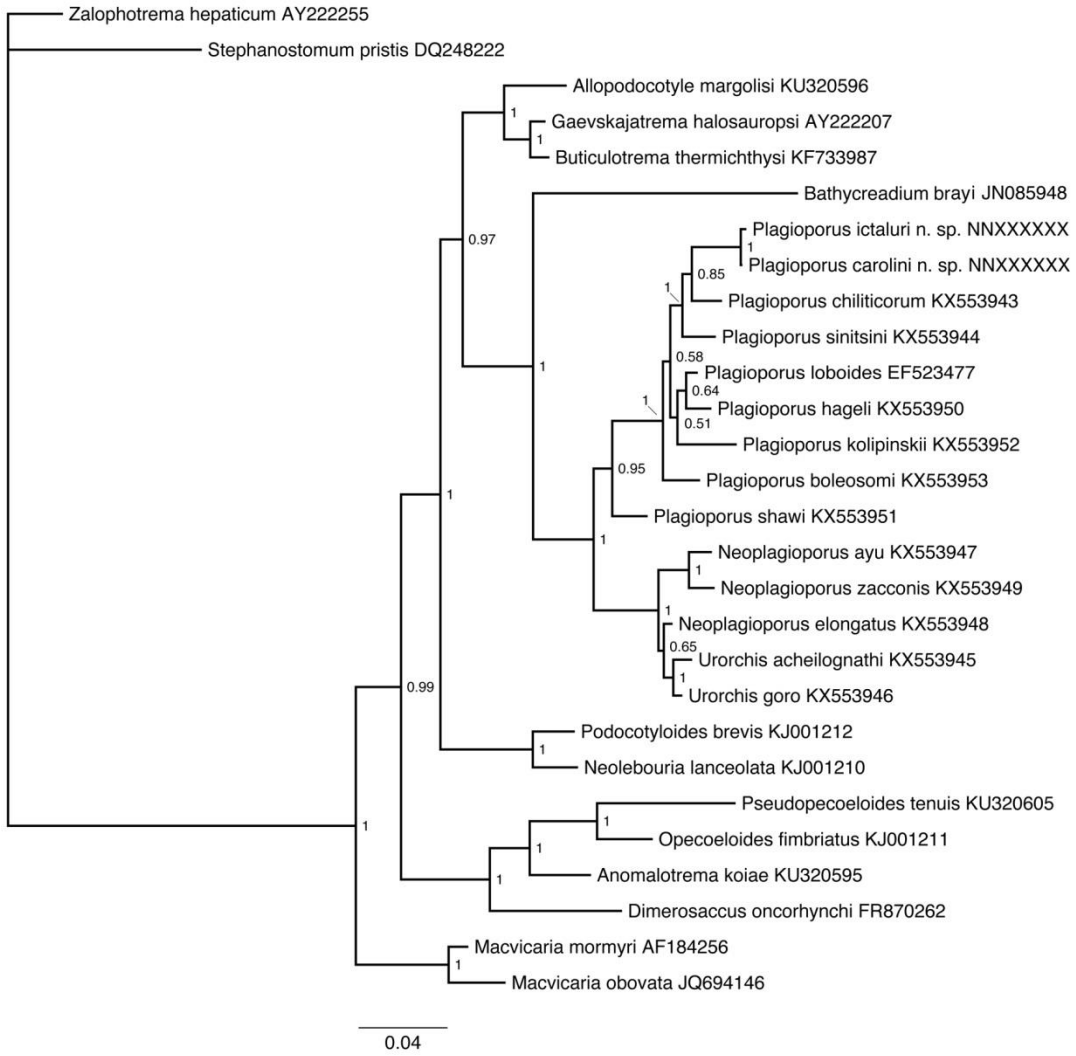


Figure 5. Phylogenetic relationships among members of *Plagioporus* resulting from Bayesian inference analysis of partial 28S rDNA sequences (GTR + I + Γ); 5,000,000 generations and a sample frequency of 1,000).



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CHAPTER IV – Three new species of *Plagioporus* Stafford, 1904 from darters (Perciformes: Percidae), with a redescription of *Plagioporus boleosomi* (Pearse, 1924) Peters, 1957

Abstract

A form of *Plagioporus* Stafford, 1904 is described from the intestine of three North American species of darters (Perciformes: Percidae) from River West Twin, Wisconsin, USA, that I consider to be conspecific with *Plagioporus boleosomi* (Pearse, 1924) Peters, 1957 based on similarities in the sucker ratio, extent of the forebody, shape and position of the testes, vitellarium distribution and terminal genitalia. Three new species of *Plagioporus* are described from the intestine of darters as follows: *Plagioporus fonti* n. sp. from *Percina nigrofasciata* Agassiz in Florida, USA, *Plagioporus limus* n. sp. from *Etheostoma squamosum* Distler in Arkansas, USA and *Plagioporus aliffi* n. sp. from *Etheostoma blennioides newmanni* Miller in Arkansas, USA. Morphologically *Plagioporus fonti* n. sp., *Plagioporus limu*s n. sp. and *Plagioporus aliffi* n. sp. are most similar to one another and to *P. boleosomi*, *Plagioporus lepomis* Dobrovolny, 1939 and '*P. etheostomae*', a *nomen nudum* for a species described from *Etheostoma blennioides* Rafinesque in Kentucky, USA, all of which are collectively distinguished from congeners in having a combination of confluent vitellarium in the post-testicular space and absence of vitelline follicles with their entire length distributed in the forebody. *Plagioporus fonti* n. sp., *P. limu*s n. sp. and *P. aliffi* n. sp. are respectively distinguished from one another and their closest congeners in having the anterior extent of the vitellarium in the anterior half of forebody to slightly anterior to the ventral sucker as opposed to one approximately at the level of the posterior margin of the ventral sucker, possession of an excretory

vesicle reaching the anterior testis as opposed to one only reaching the posterior testis and having a longer than wide oral sucker and a wider than long ventral sucker. A Bayesian inference (BI) analysis of partial 28S rDNA sequences was conducted using the three new species and 26 sequences of opecoelids retrieved from GenBank, including 10 species of *Plagioporus*. *Plagioporus aliffi* n. sp., *Plagioporus fonti* n. sp. and *P. boleosomi* comprised a moderately supported sister group to a clade containing all species of *Plagioporus* except *Plagioporus limus* n. sp. and *Plagioporus shawi* (Mcintosh, 1939) Margolis, 1970. *Plagioporus limus* and in turn *P. shawi* were resolved as sister to all other congeners with high and moderate support, respectively.

Introduction

Of the 14 valid species of *Plagioporus* Stafford, 1904 in the Nearctic region, *Plagioporus boleosomi* (Pearse, 1924) Peters, 1957 is the only species to primarily parasitize darters (Hoffman, 1999; Kuntz & Font, 1984). This species was described from *Etheostoma nigrum* Rafinesque and was also reported from *Percina caprodes* (Rafinesque) from Lake Pepin, Wisconsin, USA, by Pearse (1924). Subsequently Kuntz & Font (1984) redescribed *P. boleosomi* from *Etheostoma flabellare* Rafinesque from O'Neil Creek, Chippewa County, Wisconsin, and reported that it infects five other species of darters from the same site, with *P. caprodes*, *E. nigrum* and *Etheostoma zonale* (Cope) hosting gravid worms and *Percina maculata* (Girard) and *E. caeruleum* Storer hosting only immature worms. Outside of Wisconsin, *P. boleosomi* has been reported from several etheostomines; *Etheostoma blennioides* Rafinesque, *E. caeruleum*, *E. flabellare* and *Etheostoma spectabile* (Agassiz) in Kentucky, USA (Aliff, 1977); *Percina shumardi* (Girard) in Georgia, USA (Howard & Aliff, 1980); and *E. zonale* and *P.*

caprodes in New Hampshire, USA (Talton & Gleason, 1978). *Plagioporus boleosomi* infects several fish species in addition to darters: *Pylodictis olivaris* (Rafinesque) in Georgia, USA (Howard & Aliff, 1980); and *Sander vitreus* (Mitchill) and *Salvelinus fontinalis* (Mitchill) in Wisconsin, USA (Kuntz & Font, 1984).

The only other opecoelid reported from a darter in the Nearctic is '*Podocotyle etheostomae*' of Aliff (1973) from *E. blennioides* from North Elkhorn Creek, Kentucky, USA. While Blend & Dronen (2015) considered this form a *nomen nudum*, Aliff (1973) compared it to *P. boleosomi* and found that the two opecoelids differed in sucker ratios and relative length of the oesophagus and pharynx. Thus, there may be undocumented diversity of opecoelids in darters.

In this study, I first provide a supplemental description of *P. boleosomi* based on newly collected material from *P. caprodes, E. nigrum* and *P. maculata* from River West Twin, Wisconsin. I then describe three new species of *Plagioporus* from darters collected during a parasitological survey of freshwater fish in the southeastern USA; these comprise one resembling '*P. etheostomae*' of Aliff (1973) from *Etheostoma blennioides newmanni* Miller from Arkansas, another from Arkansas from *Etheostoma squamosum* Distler and a third from *Percina nigrofasciata* Agassiz from Florida. The new species are described using morphological information and molecular data from the ribosomal DNA gene to assess their phylogenetic relationships with *P. boleosomi* and other congeners.

Material and Methods

Specimens of *Plagioporus* were obtained from the intestine of *P. nigrofasciata* collected using a kicknet from the run of Alexander Spring, Lake County, Florida (29°4'50.82"N, 81°33'58.68"W) on 30 March 2013. Intestinal *Plagioporus* spp. were

obtained from darters during other collections using a backpack electroshocker as follows: specimens infected *E. blennioides newmanni* at Walnut Creek off Hickorynut Mountain Road, Garland County, Arkansas (34°31'59.09"N, 93°22'21.12"W) on 23 November 2014, the same host at North Big Creek at St. Highway 354, Sharp County, Arkansas (36°13'20.57"N, 91°34'50.76"W) on 8 July 2015, *E. squamosum* at Flint Creek off Fairmont Road at Springtown, Benton County, Arkansas (36º°15'9.9"N, 94°26' 25.8"W) on 19 March 2014 and *P. caprodes*, *P. maculata* and *E. nigrum* at River West Twin, Wisconsin (44°16' 10.20"N, 87°44'57.58"W) on 2 July 2009. Specimens of opecoelids were removed from the intestine of fish hosts, transferred to a shallow dish containing 0.6% saline and observed alive. Subsequently, most of the saline was removed from this dish to the point where worms were restricted to the surface of the dish and attached to the glass by their suckers. In all cases, except for the worms collected from the River West Twin for morphology, near boiling (steaming hot) water was then rapidly added to kill worms, minimizing contraction or curling post-fixation. Heat-killed worms were immediately transferred to 10% neutral phosphate-buffered formalin for morphological examination or 95% ethanol for molecular analysis. Worms collected from River West Twin were simultaneously heat-killed and fixed in steaming 10% neutral buffered formalin (3.8% formaldehyde solution) for morphological examination. Worms were stained in acetocarmine or Mayer's or Ehrlich's haematoxylin, dehydrated in a graded ethanol series, cleared in methyl salicylate and mounted permanently in Canada balsam or Damar gum. Helminth specimens collected during the present study were deposited in the collection of the Smithsonian National Museum of Natural History (NMNH), Washington, D.C. (Table 11). Specimens were examined using brightfield and

Nomarski differential interference contrast (DIC) optics on an Olympus BX 51 microscope and illustrated using an attached drawing tube. The length of the internal seminal vesicle was measured along its central axis, following its turns and loops. Measurements are given in micrometres (μm) unless otherwise specified and are expressed as the measurements of the holotype followed by the minimum and maximum values of paratypes in parentheses. The length and width of vitelline follicles are expressed as means and standard deviations of 15 random follicles distributed throughout the body. Characters expressed as a measurement followed by body length (BL) refer to the distance from the anterior end. For the supplemental description of *P. boleosomi*, minimum and maximum values of specimens are presented in Table 12 along with measurements from the original description of Pearse (1924) and the redescription of Pritchard (1966). Additional measurements were made from the line drawings of Pearse (1924) and Pritchard (1966) using the length of the holotype (no scale-bar was included with the illustration) and the provided scale-bar for scale, respectively.

Genomic DNA was isolated from each species of *Plagioporus* [number of replicates (from separate individual worms) per species displayed in Table 11] using Qiagen DNAeasy Tissue Kit (Qiagen, Inc., Valencia, California, USA) following the instructions provided by the manufacturer. DNA fragments *c*.2,550 base pairs long, comprising the 3' end of the 18S nuclear rDNA gene, internal transcribed spacer regions, ITS1 and ITS2 (including 5.8S), and a partial sequence of the 28S rRNA gene (including variable domains D1–D3), were amplified from the extracted DNA by polymerase chain reaction (PCR) on a PTC-200 Peltier Thermal Cycler using forward primer ITSF (5'-CGC CCG TCG CTA CTA CCG ATT G-3') and reverse primer 1500R (5'-GCT ATC

CTG AGG GAA ACT TCG-3'). These PCR primers and multiple internal primers were used in sequencing reactions. The internal forward primers were digl2 (5'-AAG CAT ATC ACT AAG CGG-3'), 300F (5'-CAA GTA CCG TGA GGG AAA GTT G-3') and 900F (5'-CCG TCT TGA AAC ACG GAC CAA G-3') and the internal reverse primers were 300R (5'-CAA CTT TCC CTC ACG GTA CTT G-3'), digl2R (5'-CCG CTT AGT GAT ATG CTT-3') and ECD2 (5'-CTT GGT CCG TGT TTC AAG ACG GG-3') (for primers see Littlewood et al., 2000; Tkach et al., 1999, 2000, 2001, 2003; Tkach & Snyder, 2007). The resulting PCR products were excised from PCR gels using QIAquick Gel Extraction Kit (Qiagen, Inc., Valencia, California, USA) following the manufacturer's instructions, cycle-sequenced using ABI BigDye™ chemistry (Applied Biosystems, Inc., Carlsbad, California, USA), ethanol-precipitated and run on an ABI 3130 Genetic Analyzer™. The sequences of the three new species herein described were assembled using Sequencher™ (GeneCodes Corp., Ann Arbor, Michigan, USA, Version 4.10.1) and deposited in the GenBank database (Table 11). The sequences were aligned using MAFFT version 6.611b (Katoh et al., 2005) with 1,000 cycles of iterative refinement and the genafpair algorithm. The boundaries between the 5.8S, ITS2 and 28S genes were located using the ITS2 Ribosomal Database (Keller et al., 2009). Pairwise sequence comparisons of the ITS1, 5.8S, ITS2 and 28S nuclear rDNA genes of the three new species of *Plagioporus* from this study and available sequences of *Plagioporus* from GenBank were calculated with MEGA v6 using the "compute pairwise differences function," with gaps treated using the "pairwise deletion" function. For phylogenetic analysis, sequences of opecoelids were obtained from GenBank (Table 14). The resulting alignment utilized 29 opecoelids, an acanthocolpid and used the brachycladiid

Zalophotrema hepaticum Stunkard & Alvey, 1929 as the outgroup based on its phylogenetic position relative to the Opecoelidae (see Olson et al., 2003). Phylogenetic analysis of the data was performed using Bayesian Inference (BI) with MrBayes 3.2.6 software (Huelsenbeck & Ronquist, 2001) run on the CIPRES portal (Miller et al., 2010). The best nucleotide substitution model was estimated with jModeltest-2 (Darriba et al., 2012) as general time reversible with estimates of invariant sites and gamma-distributed among site-rate variation (GTR + I + Γ). The following model parameters were used in MrBayes: nst = 6, rates = invgamma, ngen = 5,000,000 and samplefreq = 1,000. Burn-in value was 4,000 estimated by plotting the log-probabilities against generation and visualizing plateau in parameter values (sump burnin = 4,000), and nodal support was estimated by posterior probabilities (sumt) (Huelsenbeck et al., 2001) with all other settings left as default.

Resdescription of *Plagioporus boleosomi* (Pearse, 1924) Peters, 1957

Opecoelidae Ozaki, 1925

Plagioporus Stafford, 1904

Plagioporus boleosomi (Pearse, 1924) Peters, 1957

Type-host: *Etheostoma nigrum* Rafinesque, Johnny Darter (Perciformes: Percidae).

Other hosts: This study: *Percina caprodes* (Rafinesque), logperch; *Percina maculata* (Girard), blackside darter.

Locality: This study: River West Twin, Manitowoc County, Wisconsin, USA (44°16'10.20"N, 87°44'57.58"W).

Site in host: Intestine.

Voucher material: Vouchers (USNM 1416789; 1421767–1421768).

Representative DNA sequences: Partial 18S, complete ITS1 and ITS2 regions, 5.8S gene, partial (D1–D3) 28S: GenBank accession no. KX553953, from 3 identical sequences (from separate individual worms).

Description (Figs. 6.1–4)

[Measurements based on three gravid wholemounts ex *P. caprodes*.] Body white to yellow in life, elongate cylindrical, with bluntly rounded ends, tapering anteriorly, widest at approximately 1/3 to 2/5 of body length (BL), 751–906 long, 162–213 wide. Oral sucker subterminal, subequal, 86–110 × 84–109. Ventral sucker subequal, 133–182 × 142–173; width representing 81–88% of body width. Forebody 131–232, representing 17–31% of BL. Ratio of oral sucker to ventral sucker width 1:1.58–1.69. Prepharynx 18–23 long. Pharynx wider than long, slightly separated from to overlapping oral sucker by 1/2 length, 40–52 × 47–69. Oesophagus 42–84 long, representing 6–9% of BL, with or without 90° turn. Intestinal bifurcation at level of to slightly anterior to ventral sucker at 165–239 BL, representing 21–26% of BL; postcaecal space 63–78 long, representing 8–9% of BL.

Testes 2, tandem, slightly overlapping; anterior testis 89–109 × 109–123, slightly overlapping caeca ventrally, with anterior margin at 446–583 BL, representing 60–64% of BL; posterior testis 95–113 × 97–110, dorsal to anterior testis, with anterior margin at 526–664 BL, representing 70–73% of BL. Post-testicular space 103–169, representing

14–19% of BL. Cirrus-sac clavate, 120–123 long, representing 13–18% of BL, 60–69 wide, overlapping anterior 1/4–3/4 of ventral sucker. Vas deferens uniting vasa efferentia at proximal end of cirrus-sac. Internal seminal vesicle S-shaped, 116–142 long, representing 96–113% length of cirrus-sac, 44–62 wide, occupying posterior 42–73% length of sac, communicating with *pars prostatica* at 90° turn. Cirrus everted in one specimen. Ejaculatory duct present, not clearly differentiated from *pars prostatica*. Genital pore ventrolateral, sinistral, 147–212 from anterior margin of body, representing 20–23% of BL.

Ovary elongate-oval to ovoid, wider than long, 55–75 × 79–97, dextral, oblique to tandem to anterior testis, overlapping anterior testis slightly to posterior 1/3 of length, overlapping dextral caecum ventrally, with anterior margin at 407–552 BL, representing 54–61% of BL. Postovarian space 265–339, representing 34–37% of BL. Oviduct extending anterodorsally from anterior portion of ovary, turning posteriorly to join canalicular seminal receptacle; seminal receptacle median, anterodorsal to anterior testis, extending posteriorly to posterior 1/3 of ovary to anterior 1/2 of anterior testis. Laurer's canal extending dextrally from seminal receptacle, opening sinistrally on dorsal surface at level of ovary. Mehlis' gland anterior to ovary. Oötype extending anteriorly from seminal receptacle, conspicuous at level of Mehlis' gland. Uterus preovarian, containing 4–7 eggs. Metraterm overlapping posterior half of to extending slightly posterior to ventral sucker, becoming thickly muscular at level of cirrus-sac, dorsal to cirrus-sac, joining distal end of ejaculatory duct at genital pore. Eggs 71–74 × 39–45. Vitellarium follicular, mostly ventral to caeca, confined to hindbody, confluent in post-testicular space, anterior extent slightly anterior to posterior margin of ventral sucker, 246–409 from anterior end,

representing 33–45% of BL, posterior extent at 732–888, representing 96–98% of BL. Follicles of vitellarium number 95–126, length 31 ± 7, width 28 ± 7. Vitelline reservoir median, dorsal to anterior testis, ventral to seminal receptacle. Common vitelline duct joining oötype dorsally anterior to Mehlis' gland.

Excretory vesicle I-shaped, extending anteriorly to level immediately posterior to posterior testis to posterior 1/2 of posterior testis, 151–213 long, representing 20–24% of BL, 27–42 wide; pore terminal.

Remarks

Pearse (1924) described *P. boleosomi* (as *Allocreadium boleosomi* Pearse, 1924) based on seven specimens from *E. nigrum* (reported as '*Boleosoma nigrum*') and three from *P. caprodes* from Lake Pepin. However, his description is brief and provided only the measurements of the holotype that, based on his illustration, appears to have been fixed in a contracted state. Subsequently, Pritchard (1966) examined the type-material of *P. boleosomi*, which consists of a single slide containing the holotype, four paratypes and a cyst, and provided a brief redescription based on the specimens she considered to be 'in good condition,' including the holotype and two paratypes. The paratype illustrated by Pritchard (1966) is similar to the holotype in appearing to have been fixed in a contracted state. Discrepancies between Pearse's (1924) measurements of the holotype and the measurements that include those of the holotype provided by Pritchard (1966) are likely due to human error or due to the different ways in which the specimens were measured. The length and width of the holotype of *P. boleosomi* provided by Pearse (1924), for example, are 1,330 and 370 μm, respectively, whereas the length and width from the redescription of Pritchard (1966) are 1,106–1,209 and 402–442 μm, respectively.

Regardless of the discrepancies in the measurements between the original description and redescription of Pritchard (1966), both sets of measurements for the length and width are greater than those of the specimens collected from River West Twin in this study from various darter hosts that collectively range from 751–933 μm long by 162–219 μm wide. However, the length of the River West Twin specimens falls within the range of measurements provided by Kuntz & Font (1984) in their redescription of *P. boleosomi* from O'Neil Creek, Wisconsin. These authors measured 50 gravid specimens of *P. boleosomi* from *E. flabellare* that ranged between 698–1,624 μm long and 176–372 μm wide. The width of the single specimen from *E. nigrum*, that from *P. maculata*, and that of two of the specimens from *P. caprodes* from River West Twin fall within the range of body length and width reported by Kuntz & Font (1984), with a single specimen from *P. caprodes* from River West Twin being slightly narrower than the minimum width reported by these authors (162 *vs* 176 μm). The width of *P. boleosomi* by Kuntz & Font (1984) would be less than the range provided had these authors not fixed their specimens under coverslip pressure, which generally provides an overestimate of specimen width compared with specimens fixed in a relaxed state (unpublished observations). Apart from providing a rough estimate of the variation in the length and width of *P. boleosomi*, the measurements of Kuntz & Font (1984) are not included in my morphological comparison of my specimens from River West Twin with the type-material of *P. boleosomi* as coverslip pressure is known to distort the size and relative position of a range of morphological features in digeneans (Pulis et al., 2013).

Despite the difference in body length and width between the River West Twin specimens and the type-material of Pearse (1924), collectively these specimens are

morphologically similar in several features (Table 12). The holotype and two paratypes of *P. boleosomi* have a ratio of oral sucker to ventral sucker width that overlaps that of specimens from *P. caprodes* and *E. nigrum* from River West Twin (and that of the specimen from *P. maculata* from the same site is only slightly different from the type-material). The position of the intestinal bifurcation expressed as a percent of BL is nearly identical in the holotype of *P. boleosomi* and the *E. nigrum* specimen from River West Twin. The position of the intestinal bifurcation for specimens from *P. caprodes* and *P. maculata* from River West Twin was only slightly more anterior. In addition, the forebody of *P. boleosomi* expressed as a percent of BL *sensu* Pritchard (1966) is consistent with that of the specimens from River West Twin. The testes of the holotype and two paratypes of *P. boleosomi* and the specimens from River West Twin are also similar in ranging from wider than long to subequal. The anterior testis of the holotype of *P. boleosomi* occupies slightly less of the body length and width than the River West Twin specimens. The posterior testis of the holotype, conversely, falls with the range of body lengths and widths of the River West Twin specimens. The holotype of *P. boleosomi* and the specimen from *E. nigrum* from River West Twin have identical extents of the post-testicular space, with the specimens from *P. maculata* and *P. caprodes* having a slightly shorter post-testicular space. The terminal genitalia of the type-material and specimens collected in the study are also very similar. While the metraterm was not illustrated by Pearse (1924), Pritchard (1966) described the metraterm of one of the paratypes of *P. boleosomi* as well developed and having the distal end expanded. The metraterm in worms from *P. caprodes*, *P. maculata* and *E. nigrum* from River West Twin is also well developed, becoming thickly muscular distally at the level of the cirrus-sac.

Features of the cirrus-sac between the River West Twin specimens and the type-material of *P. boleosomi* are consistent, with the cirrus-sac of similar length with respect to body length and having similar length to width ratios, containing an internal seminal vesicle convoluted in an S-shape of similar length and having a short, indistinct tubular region likely representing the *pars prostatica* and ejaculatory duct. The position of the genital pore of the holotype of *P. boleosomi* and the specimen from *E. nigrum* from River West Twin is nearly identical, and the specimens from *Percina* spp. from River West Twin have slightly more posteriorly located genital pores. The ovary of the holotype and two paratypes of *P. boleosomi* and the River West Twin specimens is ovoid to elongate-oval, with that of the holotype occupying a similar percent of the body length and width as that of the specimens collected in this study. With respect to egg dimensions, Pearse (1924) measured the eggs of the holotype as 160 μm × 40 μm. The egg length reported by Pearse (1924) is clearly an error as measurements of his line drawing yielded eggs 65 μm × 37 μm and Pritchard (1966) reports egg dimensions of *P. boleosomi* as 64–85 μm × 35–45 μm. The eggs of the River West Twin specimens fall within the range of those reported by Pritchard (1966). Lastly, the anterior extent of the vitellarium is similar between the holotype of *P. boleosomi* and the River West Twin specimens, reaching the posterior third of the ventral sucker in the holotype of *P. boleosomi* compared to slightly anterior to the posterior margin of the ventral sucker in the River West Twin specimens.

There were a few considerable differences between the River West Twin specimens and the type-material of *P. boleosomi* (Table 12). One such difference includes the dimensions of the pharynx. While Pritchard (1966) describes the pharynx as rounded in her redescription, her illustration of a paratype and Pearse's (1924) illustration of the

holotype depict the pharynx as longer than wide, whereas the pharynx is wider than long in specimens from *P. caprodes, P. maculata* and *E. nigrum* from River West Twin. In addition, the position of the testis and ovary is more anterior in the holotype than in the River West Twin specimens. The differences in the shape of the pharynx and position of the testis and ovary between the type-material of *P. boleosomi* and the specimens collected in this study could be an artifact of differences in fixation; the holotype of *P. boleosomi* and the paratype illustrated by Pritchard (1966) appear to have been fixed in a contracted state as opposed to the specimens from River West Twin that were killed in a relaxed state. An additional factor that could contribute to the observed differences in the position of the testes and ovary is the apparent allometric growth of the hindbody (see elongate specimens of Kuntz & Font [1984] with short forebodies) wherein relative length of the hindbody increases with increasing body lengths. If the position of the testes and ovary does not change accordingly, the position of the ovary and testis would differ across body sizes. Additional specimens of *P. boleosomi* across all body lengths would be required to test this hypothesis. Given the potential explanations for the difference in pharynx shape and testis and ovary position and morphological similarities between the type-material of *P. boleosomi* and the specimens collected in this study, we tentatively consider the specimens collected from River West Twin to be conspecific with *P. boleosomi*.

Plagioporus boleosomi from *P. maculata* and *E. nigrum* in River West Twin, Wisconsin, were morphologically very similar to *P. boleosomi* from *P. caprodes* in the same site, with most of the measurements of the specimens from *P. maculata* and *E. nigrum* falling within the range for those from *P. caprodes* (Table 12). The measurements

of *P. boleosomi* from *P. caprodes* differed slightly from specimens from *P. maculata* as follows: narrower ventral sucker, lower oral sucker to ventral sucker width ratio, shorter and narrower posterior testis, larger and fewer eggs and more anteriorly located vitellarium. The measurements of *P. boleosomi* from *P. caprodes* differed slightly from specimens from *E. nigrum* as follows: smaller body length and width, more posterior position of the intestinal bifurcation, posterior testis narrower and more posteriorly located, smaller post-testicular space, shorter and wider cirrus-sac, wider internal seminal vesicle, longer seminal vesicle relative to cirrus-sac length, more posterior position of genital pore, shorter ovary, more extensive postovarian space and smaller and fewer eggs. In addition, the oesophagus of *P. boleosomi* was shorter in *E. nigrum*, though this difference could be an artifact of a 90° turn in the oesophagus of the specimen from *E. nigrum*. Given the low number of specimens of *P. boleosomi* available for morphological comparison from River West Twin (three from *P. caprodes* and one each from *P. maculata* and *E. nigrum*) and the slight differences between them that could be attributed to undersampling, host specific differences or a combination thereof, we consider the specimens from River West Twin from *E. nigrum*, *P. maculata* and *P. caprodes* to be conspecific.

Plagioporus boleosomi can be distinguished from *P. ictaluri* Fayton & Robison, 2017, *P. carolini* Fayton, McAllister, & Connior, 2017, *P. sinitsini* Mueller, 1934, *P. serratus* Miller, 1940, *P. serotinus* Stafford, 1904, *P. cooperi* (Hunter & Bangham, 1932) Price, 1934, *P. hypentelii* Hendrix, 1973, *P. macrouterinus* Haderlie, 1953, *P. kolipinskii* Tracey, Choudhury, Cheng & Ghosh, 2009, *P. siliculus* Sinitsin, 1931, *P. shawi* (Mcintosh, 1939) Margolis, 1970 and *P. hageli* Fayton & Andres, 2016 in having the

vitellarium restricted to the hindbody and from *P. loboides* (Curran, Overstreet & Tkach, 2007) Fayton & Andres, 2016 and *P. chiliticorum* (Barger & Esch, 1999) Cribb, 2005 in having a confluent vitelline field in the post-testicular space. *P. boleosomi* is morphologically most similar to *P. lepomis* Dobrovolny, 1939 in having an S-shaped internal seminal vesicle, vitellarium restricted to hindbody, extent of excretory vesicle, pharynx dimensions, sucker ratios, testes size and ovary size. *Plagioporus boleosomi* can be distinguished from *P. lepomis* in having vitelline follicles ventral and dorsal to the caeca as opposed to those that are only ventral to the caeca, an internal seminal vesicle that is not divided into two distinct parts by a narrow, sigmoid constriction and possession of a thickly muscular metraterm that rapidly thickens distally at the level of the cirrus-sac. Egg size might also be useful in distinguishing these two species. While Dobrovolny (1939) described the eggs of *P. lepomis* as 70–80 μm × 40–60 μm, Pritchard (1966) reported an egg size for *P. lepomis* as 80–114 μm × 51–77 μm (egg size of *P. boleosomi* is 64–85 μm × 35–45 μm). *Plagioporus boleosomi* is also morphologically similar to '*P. etheostomae*', which was described by Aliff (1973) from *E. blennioides* in North Elkhorn Creek, Fayette County, Kentucky. We agree with Blend & Dronen (2015) in considering this species a *nomen nudum*. Aliff (1973) is an unpublished PhD thesis and although this dissertation was published by UMI Dissertation Services and uploaded to ProQuest Dissertations and Theses Global database, according to article 9 section 12 of the International Code of Zoological Nomenclature, a reproduction obtained on demand of an unpublished work does not constitute a publication. While an abstract of Aliff's dissertation was published in Dissertation Abstracts International, new species were only named and not distinguished from congeners, thus relegating them to *nomen nudum*.

While Aliff (1973) placed '*P. etheostomae*' in *Podocotyle* Dujardin, 1845, a marine genus *sensu* Cribb (2005), he compared this form to *P. boleosomi* (which at the time was retained in *Podocotyle*) and found that the two species differed in sucker ratios and the relative length of the oesophagus and pharynx. Given the supplemental description provided by this study, '*P. etheostomae*' cannot be distinguished from *P. boleosomi* using the relative length of the oesophagus or pharynx. However, sucker ratios are useful in distinguishing Aliff's (1973) form from *P. boleosomi* (1:1.87 in '*P. etheostomae*' compared with 1:1.5–1.76 in *P. boleosomi*). In addition, *P. boleosomi* has a cirrus-sac overlapping the anterior 1/4–3/4 of the length of the ventral sucker as opposed to one only slightly overlapping the anterior margin of the ventral sucker as in '*P. etheostomae*'. Unfortunately, Aliff (1973) fixed '*P. etheostomae*' under 'slight' coverslip pressure; thus, morphological comparisons of this form with *P. boleosomi* and other species of *Plagioporus* will have to be reassessed with freshly collected material fixed in a relaxed state.

Description of *Plagioporus fonti* n. sp.

Plagioporus fonti n. sp.

Type-host: *Percina nigrofasciata* Agassiz, Blackbanded Darter (Perciformes: Percidae).

Type-locality: Run of Alexander Spring, Lake County, Florida, USA (29°4'50.82"N, 81°33'58.68"W).

Site in host: Intestine.

Prevalence and intensity: 4 of 4 hosts (100%); 2–6 worms per host (mean 3).

Type-material: Holotype (USNM 1421761); paratype (USNM 1421762-1421764).

Representative DNA sequences: Partial ITS1 and complete ITS2 regions, 5.8S gene, partial (D1–D3) 28S: GenBank accession no. KX905054, from 3 identical sequences (from separate individual worms).

Etymology: This species is named after Dr. William F. Font (Southeastern Louisiana University, Hammond, LA) in recognition of his past and continuing contributions to parasitology and his previous work on *P. boleosomi*.

Description (Figs. 7.5–8)

[Measurements based on 10 gravid wholemounts from *P. nigrofasciata*] Body white to yellow in life, elongate cylindrical, with bluntly rounded ends, tapering anteriorly, widest at approximately 1/5 to 1/3 of body length (BL), 877 (550–914) long, 214 (148–252) wide. Oral sucker subterminal to nearly terminal, subequal, 105 × 95 (51–107 × 66–97). Ventral sucker wider than long, 136 × 145 (92–136 × 100–163); width representing 68 (65–76)% of body width. Forebody 279 (124–282), representing 32 (22–33)% of BL. Ratio of oral sucker to ventral sucker width 1:1.5 (1:1.5–2.0). Prepharynx 17 (0–24) long. Pharynx subequal, slightly separated from to slightly overlapping oral sucker, 47 × 55 (33–50 × 33–61). Oesophagus 80 (38–84) long, representing 9 (7–10)% of BL, with or without 90° turn. Intestinal bifurcation slightly anterior to ventral sucker at 246 (131–251), representing 28 (21–30)% of BL; postcaecal space 88 (42–96) long, representing 10 (8–15)% of BL.

Testes 2, tandem; anterior testis subequal, 90 × 98 (69–140 × 78–132), slightly overlapping sinistral or dextral arm of caeca ventrally, with anterior margin at 558 (335–

571) BL, representing 64 (58–65)% of BL; posterior testis subequal, 90 × 111 (82–145 × 81–129), dorsal to anterior testis, overlapping anterior testis slightly to by 1/3 of length, with anterior margin at 639 (400–647) BL, representing 73 (65–75)% of BL. Post-testicular space 142 (72–156), representing 16 (12–17)% of BL. Cirrus-sac clavate, 169 (89–153) long, representing 19 (14–20)% of BL, 71 (31–61) wide, overlapping anterior 1/2–3/4 of ventral sucker. Vas deferens uniting vasa efferentia at proximal end of cirrus-sac. Internal seminal vesicle convoluted, S-shaped, 207 (79–183) long, representing 122 (89–153)% length of cirrus-sac, 50 (22–44) wide, occupying posterior 60 (63–79)% length of sac, communicating with indistinct tubular region (likely representing *pars prostatica* and ejaculatory duct) at 90° turn. Genital pore ventrolateral, sinistral, 223 (103–208) from anterior margin of body, representing 25 (18–25)% of BL.

Ovary elongate oval, ovoid, to triangular, subequal, 83 × 73 (59–75 × 55–93), oblique to tandem to anterior testis, overlapping anterior testis in posterior 1/3 to 1/2 of length, ventrally overlapping to contiguous with dextral caecum, with anterior margin at 501 (295–548) BL, representing 57 (54–59)% of BL. Postovarian space 279 (186–303), representing 32 (31–35)% of BL. Oviduct extending anterodorsally from anterior portion of ovary, turning posteriorly to join with canalicular seminal receptacle; seminal receptacle median, dorsal to anterior testis, extending posteriorly to anterior 1/2 of anterior testis. Laurer's canal extending anterodextrally from seminal receptacle with or without turn at distal end, opening sinistrally on dorsal surface slightly anterior to ovary. Mehlis' gland median, anterior to ovary. Oötype extending anteriorly from seminal receptacle, conspicuous at level of Mehlis' gland. Uterus preovarian to extending posteriorly to anterior 2/5 ovary length, containing 1 (1–7) eggs. Metraterm arising

slightly posterior to ventral sucker, weakly muscular, dorsal to cirrus-sac, joining distal end of ejaculatory duct at genital pore. Eggs 77 × 47 (56–78 × 36–52). Vitellarium follicular, mostly ventral to caeca, almost confluent to confluent in post-testicular space, anterior extent in anterior half of to slightly anterior to ventral sucker, 332 (158–266) from anterior end, representing 38 (26–36)% of BL, posterior extent at 850 (540–896), representing 97 (97–99)% of BL. Follicles of vitellarium number 54 (51–66), length (n = 15) 40 (35 ± 5, 27–44), width (n = 15) 30 (31 ± 4, 25–34). Vitelline reservoir median, dorsal to anterior testis, ventral to seminal receptacle. Common vitelline duct conspicuous.

Excretory vesicle I-shaped, extending anteriorly to level immediately posterior to posterior testis to posterior 2/3 of posterior testis, 138 (94–166) long, representing 16 (16–24)% of BL, 35 (21–45) wide; pore terminal.

Remarks

Plagioporus fonti n. sp. can be distinguished from *P. ictaluri*, *P. carolini*, *P. cooperi*, *P. hageli*, *P. hypentelii*, *P. kolipinskii*, *P. macrouterinus*, *P. serotinus*, *P. serratus*, *P. shawi*, *P. siliculus* and *P. sinitsini* in having the vitellarium almost entirely distributed in the hindbody with only 1–2 follicles reaching anteriorly into the forebody (the 1–2 follicles are only partially distributed in the forebody; a portion of the length of these follicles overlaps the ventral sucker); from *P. loboides* in lacking a uterus that extends to the end of the body; and from *P. chiliticorum* in lacking a bipartite internal seminal vesicle. *Plagioporus fonti* n. sp. is most morphologically similar to *P. lepomis* and *P. boleosomi* in having the vitellarium almost entirely distributed in the hindbody, forming a confluent field in the post-testicular space, and in possession of a S-shaped

internal seminal vesicle. *Plagioporus fonti* n. sp. can be distinguished from *P. lepomis* in having vitelline follicles ventral and dorsal to the caeca as opposed to those that are only ventral to the caeca, an internal seminal vesicle that is not divided into two distinct parts by a narrow, sigmoid constriction, possession of a narrower body despite overlapping body lengths (148–252 μm as opposed to 280–490 μm) and in having consistently tandem testes as opposed to those that are tandem to oblique. *Plagioporus fonti* n. sp. can be further distinguished from *P. lepomis* and from *P. boleosomi* in the anterior extent of the vitellarium (the midpoint of to slightly anterior to the ventral sucker as opposed to approximately the posterior margin of the ventral sucker). *Plagioporus fonti* n. sp. is further distinguished from *P. boleosomi* in having a weakly muscular metraterm of uniform thickness at the level of cirrus-sac as opposed to a strongly muscular metraterm that rapidly thickens at the level of the cirrus-sac, possession of a wider than long ventral sucker as opposed to one that is subequal and having fewer vitelline follicles (51–66 *vs* 95–126). *Plagioporus fonti* n. sp. is similar to '*P. etheostomae*' of Aliff (1973) but is distinct in having the anterior extent of the vitellarium at the midpoint of to slightly anterior to the ventral sucker compared with one in the posterior third of the acetabulum, a forebody representing 22–33% BL *vs* 20% BL, a ventral sucker representing 65–76% of body width as opposed to 80–85% of body width and in having a cirrus-sac overlapping the anterior 1/2–3/4 of the length of the ventral sucker as opposed to one only slightly overlapping the anterior margin of the ventral sucker. *Plagioporus fonti* n. sp. is the only species of *Plagioporus* known to infect *Percina nigrofasciata* and is also the first species of its genus to be described from Florida.

Description of *Plagioporus limus* n. sp.

Plagioporus limus n. sp**.**

Type-host: *Etheostoma squamosum* Distler, Plateau Darter (Perciformes: Percidae).

Type-locality: Flint Creek off Fairmont Road at Springtown, Benton County, Arkansas, USA (36º 15’ 9.9” N, 94º, 26’ 25.8” W)

Site in host: Intestine.

Prevalence and intensity: 4 of 6 hosts (67%); 4–10 worms (mean 6).

Type-material: Holotype (USNM 1421759); paratype (USNM 1421760).

Representative DNA sequences: Partial ITS1 and complete ITS2 regions, 5.8S gene, partial (D1–D3) 28S: GenBank accession no. KX905055, from 3 identical sequences (from separate individual worms).

Etymology: The Latin adjectival name *limus* refers to condition of the testes that is unique to this species in being consistently oblique as opposed tandem as in other forms of *Plagioporus* from darters. The specific epithet was given in the form of an adjective to agree with the masculine genus name, which is a mixture of the Greek Plagio (oblique) and the Latin porus. We presume the genus name was intended to be a noun representing an oblique genital pore.

Description (Figs. 8.9–12)

[Measurements based on nine gravid wholemounts from *E. squamosum*] Body white to yellow in life, lanceolate to elongate cylindrical, with bluntly rounded ends, tapering

anteriorly, widest at approximately 1/3 to 2/3 of body length (BL), 1,110 (712–1,040) long, 318 (203–285) wide. Oral sucker subterminal, subequal, 122 × 124 (80–142 × 83–117). Ventral sucker subequal, 177 × 193 (135–187 × 135–203); width representing 61 (60–71)% of body width. Forebody 290 (183–273), representing 26 (23–30)% of BL. Ratio of oral sucker to ventral sucker width 1:1.6 (1:1.4–1.7). Prepharynx 27 (14–27) long. Pharynx subequal, slightly separated from to overlapping oral sucker by 1/3 length, 64 × 70 (38–80 × 48-60). Oesophagus 75 (31–107) long, representing 7 (3–11)% of BL. Intestinal bifurcation slightly anterior to ventral sucker at 263 (184–249) BL, representing 24 (22–27)% of BL; postcaecal space 124 (43–110) long, representing 11 (4–11)% of BL.

Testes 2, oblique to nearly parallel in one specimen; anterior testis subequal, 162 × 166 (90–140 × 76–138), slightly overlapping sinistral arm of caeca ventrally, with anterior margin at 625 (449–612) BL, representing 56 (54–63)% of BL; posterior testis subequal, 172 × 170 (98–148 × 83–169), dorsal to anterior testis, ventral to caeca, overlapping anterior testis by 1/4 to 3/4 of length, with anterior margin at 775 (474–671) BL, representing 70 (64–68)% of BL. Post-testicular space 194 (163–271), representing 17 (18–33)% of BL. Cirrus-sac clavate, 157 (121–171) long, representing 14 (14–18)% of BL, 82 (43–73) wide, overlapping anterior 1/3–3/4 of ventral sucker. Vas deferens uniting vasa efferentia at proximal end of cirrus-sac. Internal seminal vesicle S-shaped to convoluted with 3 turns, 258 (124–219) long, representing 164 (102–144)% length of cirrus-sac, 61 (38–54) wide, occupying posterior 81 (60–79)% length of sac, communicating with indistinct tubular region (likely representing *pars prostatica* and

ejaculatory duct) at 90° turn. Genital pore ventrolateral, sinistral, 239 (154–234) from anterior margin of body, representing 22 (19–24)% of BL.

Ovary elongate oval to ovoid, subequal, median to dextral, 134 × 120 (61–127 × 69–101), ventrally oblique to anterior testis, overlapping anterior testis in posterior 33–87% of length, ventrally overlapping to contiguous with dextral caecum, occasionally contiguous with sinistral caecum, with anterior margin at 608 (411–562) BL, representing 55 (52–59)% of BL. Postovarian space 370 (137–415), representing 33 (19–40)% of BL. Oviduct extending anterodorsally from dextral portion of ovary, turning posteriorly to join with canalicular seminal receptacle; seminal receptacle median, dorsal to anterior testis, extending posteriorly to posterior margin of ovary. Laurer's canal extending anterodextrally from seminal receptacle with or without turn at distal end, opening sinistrally on dorsal surface at level of anterior 1/2 of ovary. Mehlis' gland median, slightly overlapping ovary. Oötype extending anteriorly from seminal receptacle, conspicuous at level of Mehlis' gland. Uterus preovarian to extending posteriorly to anterior 1/4 ovary length, containing 8 (4 ± 2, 1–8) eggs. Metraterm arising in posterior half of to slightly posterior to ventral sucker, weakly muscular, dorsal to cirrus-sac, joining distal end of ejaculatory duct at genital pore. Eggs 74 × 44 (63–78 × 31–46). Vitellarium follicular, fields dorsal and ventral to caeca, confluent in post-testicular space, anterior extent in posterior 1/3 of ventral sucker to slightly anterior to posterior margin of ventral sucker, 415 (276–410) from anterior end, representing 37 (36–44)% of BL, posterior extent at 1,080 (676–962), representing 97 (93–99)% of BL. Follicles of vitellarium number 90 (85–112), length (n = 15) 47 (33 ± 8, 24–44), width (n = 15) 43

(31 ± 6, 24–36). Vitelline reservoir median, dorsal to anterior testis, ventral to seminal receptacle. Common vitelline duct joining oötype dorsally anterior to Mehlis' gland.

Excretory vesicle I-shaped, extending anteriorly at least to posterior margin of anterior testis and at most to midpoint of anterior testis nearly to ovary, 339 (199–376) long, representing 31 (28–36)% of BL, 127 (52–126) wide; pore terminal.

Remarks

Plagioporus limus n. sp. can be distinguished from *P. ictaluri*, *P. carolini*, *P. cooperi*, *P. hageli*, *P. hypentelii*, *P. kolipinskii*, *P. macrouterinus*, *P. serotinus*, *P. serratus*, *P. shawi*, *P. siliculus* and *P. sinitsini* in having the vitellarium restricted to the hindbody and from *P. loboides* and *P. chiliticorum* in having a confluent vitelline field in the post-testicular space. *Plagioporus limus* n. sp. is morphologically most similar to *P. lepomis*, *P. boleosomi* and *P. fonti* in sucker ratios, possession of a convoluted internal seminal vesicle, cirrus-sac length expressed as a percent of body length, vitellarium restricted or almost entirely restricted to the hindbody and ovary and testes size. *Plagioporus limus* n. sp. can be readily distinguished from these congeners in possession well-defined ventral and dorsal vitelline fields (*P. boleosomi* and *P. fonti* only have sparse vitelline follicles dorsal to the caeca; *P. lepomis* lacks a dorsal vitelline field), in having consistently oblique testes (*P. boleosomi* and *P. fonti* have tandem testes; *P. lepomis* has testes that are slightly oblique to tandem) and in having an excretory vesicle that extends at least to the level of the anterior testis that constitutes 28–36% of BL [anterior extent of the excretory vesicle in *P. boleosom*i, *P. fonti* and *P. lepomis* is the level of the posterior testis at 19-25% BL, 16–24% BL and approximately 19% (from holotype illustration), respectively]. *Plagioporus limus* n. sp. is also morphologically

similar to '*P. etheostomae*' of Aliff (1973), but can be distinguished in having an excretory bladder that extends to the level of the anterior testis as opposed to one at the level of the posterior testis, a ventral sucker representing 60–71% of body width as opposed to 80–85% of body width, an oral to ventral sucker width ratio of 1:1.4–1.7 *vs* 1:1.9 and in having a cirrus-sac overlapping the anterior 1/3–3/4 of the length of the ventral sucker as opposed to one slightly overlapping the anterior margin of the ventral sucker. *Plagioporus limus* n. sp. is the only species of *Plagioporus* known to infect *E. squamosum*.

Description of *Plagioporus aliffi* n. sp.

Plagioporus aliffi n. sp.

Type-host: *Etheostoma blennioides newmanni* Miller, Greenside Darter (Perciformes: Percidae).

Type-locality: North Big Creek at St. Highway 354, Sharp County, Arkansas, USA (36° 13' 20.57"N, 91° 34' 50.76"W)

Other locality: Walnut Creek off Hickorynut Mountain Road, Garland County, Arkansas, USA (34° 31' 59.09"N, 93° 22' 21.12"W)

Site in host: Intestine.

Prevalence and intensity: 5 of 7 hosts (71%); 1–8 worms per host (mean 3).

Type-material: Holotype (USNM 1421765); paratype (USNM 1421766).

Representative DNA sequences: Partial ITS1 and complete ITS2 regions, 5.8S gene, partial (D1–D3) 28S: GenBank accession no. KX905056, from 3 identical sequences (from separate individual worms).

Etymology: This species is named after Dr. John Vincent Aliff in recognition of his contributions to the field of parasitology, including his research on opecoelids from freshwater hosts of the Nearctic.

Description (Figs. 9.13–16)

[Measurements based on 9 gravid wholemounts from *E. b. newmanni*.] Body white to yellow in life, elongate cylindrical with bluntly rounded ends, tapering anteriorly, widest at approximately 1/4 body length (BL), 1,425 (937–1,663) long, 270 (183–313) wide. Oral sucker subterminal to nearly terminal, longer than wide, 116 × 105 (79–128 × 65–108). Ventral sucker wider than long, 178 × 203 (133–193 × 140–219); width representing 75 (69–84)% of body width. Forebody 295 (183–375), representing 21 (15–26)% of BL. Ratio of oral sucker to ventral sucker width 1:1.9 (1:1.9–2.3). Prepharynx 9 (9–26) long. Pharynx subequal, slightly separated from to overlapping oral sucker by 1/3 length, 61 × 67 (35–62 × 41–71). Oesophagus 108 (82–140) long, representing 8 (7–9)% of BL, with or without 90° turn. Intestinal bifurcation at level of to slightly anterior to ventral sucker at 274 (194–336) BL, representing 19 (17–22)% of BL; postcaecal space 86 (54–121) long, representing 6 (5–9)% of BL.

Testes 2, tandem, contiguous to separated by distance of 38 (25–42), representing 3 (2–3)% of BL; anterior testis subequal to nearly round, 118 × 114 (76–146 × 77–136), slightly overlapping arms of caeca ventrally, with anterior margin at 846 (631–1,060) BL, representing 59 (58–67)% of BL; posterior testis subequal to nearly round, 135 × 123 (89–155 × 87–142), dorsal to anterior testis, overlapping to contiguous with arms of

caeca, with anterior margin at 996 (702–1,223) BL, representing 70 (67–75)% of BL. Post-testicular space 299 (138–380), representing 21 (15–23)% of BL. Cirrus-sac clavate to elongate ovoid, 166 (99–208) long, representing 12 (10–13)% of BL, 62 (50–77) wide, with posterior end reaching posteriorly to posterior margin of ventral sucker to anterior 1/3 ventral sucker. Vas deferens uniting vasa efferentia at proximal end of cirrus-sac. Internal seminal vesicle convoluted with 3 turns, 205 (145–326) long, representing 123 (129–191)% length of cirrus-sac, 33 (22–62) wide, occupying posterior 67 (56–75)% length of sac, communicating with indistinct tubular region (likely representing *pars prostatica* and ejaculatory duct) at 90° turn. Genital pore ventrolateral, sinistral, 268 (176–305) from anterior margin of body, representing 19 (13–21)% of BL.

Ovary ovoid, subequal, median to submedian, 89 × 96 (47–92 × 52–90), slightly overlapping anterior testis ventrally, ventrally overlapping to contiguous with sinistral caecum, with anterior margin at 773 (596–968) BL, representing 54 (51–64)% of BL. Postovarian space 578 (296–690), representing 41 (32–43)% of BL. Oviduct extending anterodorsally from dextral portion of ovary, turning posteriorly to join with canalicular seminal receptacle; seminal receptacle median, dorsal to ovary, extending slightly anterior to ovary. Laurer's canal extending laterally to posteriorly from seminal receptacle, turning anteriorly before sinistral to median opening on dorsal surface at level of to slightly anterior to ovary, with or without turn at distal end. Mehlis' gland median, anterior to ovary. Oötype extending anteriorly from seminal receptacle, conspicuous at level of Mehlis' gland. Uterus preovarian, containing 5 (1–16) eggs. Metraterm arising in posterior half of to slightly posterior to ventral sucker, weakly muscular, dorsal to cirrus-sac, joining distal end of ejaculatory duct at genital pore. Eggs 69 × 43 (62–69 × 37–47).

Vitellarium follicular, ventral to caeca with sparse field dorsal to caeca, confluent in post-testicular space, anterior extent slightly posterior to slightly anterior to posterior margin ventral sucker, 454 (352–593) from anterior end, representing 32 (25–39)% of BL, posterior extent at 1,390 (919–1,675), representing 98 (98–100)% of BL. Follicles of vitellarium number 139 (101–141), length (n = 15) 34 (32 ± 7, 19–41), width (n = 15) 30 (29 ± 5, 22–35). Vitelline reservoir median, dorsal to anterior testis, ventral to seminal receptacle. Common vitelline duct joining oötype dorsally anterior to Mehlis' gland.

Excretory vesicle I-shaped, extending anteriorly to slightly posterior to posterior testis to nearly to anterior testis, 324 (168–381) long, representing 23 (18–23)% of BL, 47 (25–93) wide; pore terminal.

Remarks

Plagioporus aliffi n. sp. can be distinguished from *P. carolini*, *P. ictaluri*, *P. cooperi*, *P. hageli*, *P. hypentelii*, *P. kolipinskii*, *P. macrouterinus*, *P. serotinus*, *P. serratus*, *P. shawi*, *P. siliculus* and *P. sinitsini* in having the vitellarium restricted to the hindbody and from *P. loboides* and *P. chiliticorum* in having a confluent vitelline field in the post-testicular space. *Plagioporus aliffi* n. sp. is morphologically most similar to *P. lepomis*, *P. boleosomi*, *P. fonti* and *P. limus* in possessing a convoluted internal seminal vesicle, cirrus-sac length expressed as a percent of body length and in having the vitellarium restricted or almost entirely restricted to the hindbody. The new species can be distinguished from these congeners in having a combination of a longer than wide oral sucker and a wider than long ventral sucker; oral and ventral suckers are subequal in *P. lepomis*, *P. boleosomi* and *P. limus*, whereas *P. fonti* has a wider than long ventral sucker but a subequal oral sucker. *Plagioporus aliffi* n. sp. can be further distinguished from *P.*

lepomis in having vitelline fields ventral and dorsal to the caeca as opposed to only those that are ventral to the caeca and in the absence of an internal seminal vesicle that is divided into two distinct parts by a narrow, sigmoid constriction; from *P. boleosomi* in having a weakly muscular metraterm that does not thicken distally at the level of the cirrus-sac, an internal seminal vesicle representing 123–191% *vs* 91–113% of the length of the cirrus-sac and an esophagus representing 34–47% as opposed to 14–32% of forebody length; from *P. fonti* in having a more posterior position of the anterior extent of the vitellarium (approximately at the level of the posterior margin of the ventral sucker compared with the anterior 1/2 of to slightly anterior to the ventral sucker), body size (937–1,663 *vs* 550–914 μm) and in possession of fewer vitelline follicles (101–141 *vs* 51–66); and from *P. limus* in the excretory vesicle reaching the level of the posterior testis or ending slightly posterior to it as opposed to reaching the anterior testis and in having tandem *vs* consistently oblique testes. *Plagioporus aliffi* n. sp. is further distinguished from *P. boleosomi*, *P. limus* and *P. lepomis* in the ratio of the width of the oral sucker to ventral sucker width [1:1.9–2.3 compared with 1:1.6–1.8, 1:1.4–1.7, and 1:1.7 (from holotype illustration), respectively]. *Plagioporus aliffi* n. sp. is also morphologically similar to '*P. etheostomae*' of Aliff (1973); both opecoelids are from *E. blennioide*s and have the vitellarium restricted to the hindbody, oral sucker to ventral sucker width ratios and body lengths that overlap, and subequal to nearly round testes that are contiguous or separated by a short distance. As previously noted, Aliff (1973) fixed his specimens under slight coverslip pressure, making comparisons with my material tenuous. The illustrations of '*P. etheostomae*' depict a ventral sucker that is wider than long but not an oral sucker that is longer than wide, rather one that is

subequal. Moreover, the oesophagus of *Plagioporus aliffi* n. sp. is longer than that of '*P. etheostomae*' (82–140 *vs* 46–71 μm) and this in turn alters the position of the intestinal bifurcation (at the level of to slightly anterior to the anterior margin of the ventral sucker in *Plagioporus aliffi* n. sp. as opposed to the midpoint between the posterior margin of the pharynx and the anterior margin of the ventral sucker in '*P. etheostomae*'). *Plagioporus aliffi* n. sp. further diverges from '*P. etheostomae*' in having the cirrus-sac overlap the ventral sucker by 1/3 to all of the ventral sucker length compared with a cirrus-sac that only slightly overlaps the anterior margin of the ventral sucker. To ascertain whether these differences could be an artifact of fixation, three specimens of *Plagioporus aliffi* n. sp. were fixed under slight coverslip pressure. We found that this slight coverslip pressure did not alter significantly any of the above differences between *Plagioporus aliffi* n. sp. and '*P. etheostomae*' and regard these two forms as distinct species. '*P. etheostomae*' was reported from over 724 km away from the type-locality of *Plagioporus aliffi* n. sp.

Molecular Analysis

No intraspecific variation was observed for *Plagioporus fonti* n. sp., *Plagioporus aliffi* n. sp. and *Plagioporus limus* n. sp. (Table 11). Sequence lengths of the ITS1 rDNA gene for *Plagioporus fonti* n. sp., *Plagioporus limus* n. sp. and *Plagioporus aliffi* n. sp. were 671, 651 and 671 bp, respectively, with that of *Plagioporus limus* n. sp. being the only incomplete ITS1 sequence. The length of the complete 5.8S rDNA gene for all of these species was 156 bp and lengths of the partial 28S rRNA gene fragment ranged between 1,227–1,230 bp (when trimmed to the shortest sequence in the alignment for base pair comparisons, *P. loboides*). The length of the complete ITS2 fragment was 250

bp for the new species of *Plagioporus* except for *Plagioporus aliffi* n. sp., which had a length of 251 bp.

In the partial 28S, complete ITS2 and complete ITS1 rDNA sequences, *P. boleosomi*, *Plagioporus aliffi* n. sp. and *Plagioporus fonti* n. sp. were closely related. For the same loci, *Plagioporus limus* n. sp. was not more closely related to the other species from darters collectively than it was to other congeners. Variation between species in the complete 5.8S rDNA gene was minimal (Table 13).

The alignment of the partial 28S rRNA gene sequences of the 3 new species and those of other opecoelids from GenBank was 1,284 bp with 791 conserved, 484 variable and 348 informative sites. The plagioporines from freshwater hosts were divided into 2 clades; one consisted of species from the Palearctic (*Urorchis* Ozaki, 1925 and *Neoplagioporus* Shimazu, 1990 from Japan) and the sister clade consisted of *Plagioporus* spp. from the Nearctic. *Plagioporus shawi* and in turn *Plagioporus limus* n. sp. were resolved as sister (with moderate and high support, respectively) to a clade containing all other species of *Plagioporus*. This clade consisted of one containing *P. boleosomi*, *Plagioporus fonti* n. sp. and *Plagioporus aliffi* n. sp. (with *P. boeosomi* being sister to *Plagioporus fonti* n. sp and in turn sister to *Plagioporus aliffi* n. sp., all with high support) resolved as the sister to another clade with moderate support. The clade sister to that containing the 3 species of *Plagioporus* from darters was divided with low support into one containing *P. sinitsini*, *P. chiliticorum*, *P. ictaluri* and *P. carolini*, the interrelationships of which were all resolved with high support, and another in which *P. hageli* was resolved as the highly supported sister to *P. kolipinskii* and *P. loboides*, which were sister to one another with low support (Fig 10). The phylogenetic position of

freshwater plagioporines is consistent with that of Fayton & Andres (2016) and Bray et al. (2016).

Discussion

Morphologically the species of *Plagioporus* from darters, *P. boleosomi*, *Plagioporus fonti* n. sp., *Plagioporus aliffi* n. sp. and *Plagioporus limus* n. sp. were most similar to one another and also to a form '*P. etheostomae*' (*nomen nudum*) of Aliff (1973) from *E. blennioides* from Kentucky and to *P. lepomis*. The BI analysis of partial 28S rDNA confirmed that *P. boleosomi*, *Plagioporus fonti* n. sp. and *Plagioporus aliffi* n. sp. are more closely related to one another than they are to their other congeners. Given that these three species from darters are biogeographically disparate (occurring in Wisconsin, Arkansas and Florida), with their type-localities being closer to those of congeners not occurring in darters than they are to one another, and their morphological and molecular similarity, I hypothesize that *Plagioporus* has experienced a host switching event between an unknown freshwater host and an etheostomine that was followed by a radiation of *Plagioporus* across percid hosts in the eastern Nearctic. Biogeographically, *P. boleosomi* occurs sympatrically with *P. sinitsini* in River West Twin, Wisconsin (unpublished data); *Plagioporus aliffi* n. sp. is distributed in northeastern Arkansas along with forms of *Plagioporus* from cottids and ictalurids that likely represent new species (McAllister et al., 2014a, b; 2015); of its congeners *Plagioporus fonti* n. sp. occurs closest to *P. loboides*. *Plagioporus limus* n. sp. may be a part of this radiation or alternatively it may represent a second independent radiation into darter hosts. While *Plagioporus limus* n. sp. is morphologically similar to its congeners from darter hosts, it is apparently not more related to these species collectively than it is to other congeners;

my BI analysis resolved it as sister with high support to all other species of *Plagioporus* except *P. shawi*. The inclusion of additional species of *Plagioporus* from percid hosts and *P. lepomis* in future phylogenies may clarify the placement of *Plagioporus limus* n. sp. within *Plagioporus*. While the relationship between *Plagioporus limus* n. sp. and its congeners remains unclear, its nesting within *Plagioporus* confirms that the excretory bladder of species of *Plagioporus*, as suggested by Fayton & Andres (2016), can extend as far anteriorly to the level of the anterior testis nearly to the ovary.

The supplemental description of *P. boleosomi* was based on specimens from River West Twin, Wisconsin, which is part of the Lake Michigan drainage approximately 322 km away and hydrologically disjunct from the type-locality of *P. boleosomi*, Lake Pepin. Specimens from River West Twin exhibited slight variation from the original description and these were attributed to the contracted nature of Pearse's (1924) specimens and the apparent allometric growth of the hindbody in *P. boleosomi*. I considered the specimens from River West Twin to be conspecific with *P. boleosomi* based on several morphological traits shared between my material, Pearse's (1924) description and Pritchard's (1966) redescription. The rapid thickening of the metraterm at the level of the cirrus-sac may be a character unique to *P. boleosomi*; such a distal thickening of the metraterm was not observed in the three new species from darters described in this study. Future studies on *Plagioporus* from darter hosts should include the sequencing of *P. boleosomi* from its type-locality and host along with a redescription based on measurements from specimens of a range of body lengths. In addition to *P. boleosomi*, '*P. etheostomae*' of Aliff (1973) would be a useful species to describe given that the specimens prepared by Aliff (1973) were fixed under slight coverslip pressure, its

nomen nudum status and its biogeographical separation from other forms of *Plagioporus* parasitizing darters. Based on information presented in this study, I hypothesize that a phylogenic analysis based on DNA sequence data that includes '*P. etheostomae*' will confirm this form as the sister species of *Plagioporus aliffi* n. sp. given their morphological similarity and common host, *E. blennioides*.

Table 11 Species of *Plagioporus* collected from the Nearctic and their respective hosts, collection localities, GenBank accession number (with number of replicates in parenthesis) and deposition information.

Species	**Host**	**Locality**	**Date**	**GenBank (number of replicates in parenthesis)**	**NMNH**
Plagioporus boleosomi (Pearse, 1924) Peters, 1957	*Percina caprodes* Rafinesque	River West Twin, W.I., USA	2/20/2009	–	1421768
	Percina maculata Girard	River West Twin, W.I., USA	2/20/2009	KX553953 (3)	1416789
	Etheostoma nigrum Rafinesque	River West Twin, W.I., USA	2/20/2009	–	1421767
Plagioporus fonti n. sp.	*Percina nigrofasciata* Agassiz	Alexander Spring, F.L., USA	3/30/2013	KX905054 (3)	1421761–4
Plagioporus limus n. sp.	*Etheostoma squamosum* Distler	Flint Creek, A.R., USA	3/30/2014	KX905055 (3)	1421759–60
Plagioporus aliffi n. sp.	*Etheostoma blennioides*	North Big Creek, A.R., USA	07/082015	KX905056 (3)	1421765–6
	newmanni Miller	Walnut Creek, A.R., USA	6/23/2014	–	–

Table 12 Measurements of *Plagioporus boleosomi* from Pearse (1924), Pritchard (1966) and those from this study from *Percina caprodes*, *Percina maculata* and *Etheostoma nigrum* from River West Twin, Wisconsin

Host Source	*Percina caprodes* Present study (n = 3)	*Percina maculata* Present study (n = 1)	*Etheostoma nigrum* Present study (n=1)	Holotype from *E. nigrum* Pearse (1924)	Redescription of holotype by Pritchard (1966) (n = 3)
Body length	751–906	881	933	1,330	1,106–1,209
Body width	162–213	205	219	370	402–442
Oral sucker length	86–110	106	97	140	–
Oral sucker width	84–109	102	97	140	127–160
Ventral sucker length	133–182	165	158	220	–
Ventral sucker width	142–173	180	157	220	214–241
Oral sucker to ventral sucker width ratio	1:1.58–1.69	1:1.76	1:1.62	1:1.57	1:5.–1.7
Pharynx length	40–52	43	40	74[a]	–
Pharynx width	47–69	61	56	58[a]	–
Oesophagus length	42–84	80	27	–	–
Intestinal bifurcation as % BL	21–26	22	18	17[a]	–
Postcaecal space as % BL	8–9	9.0	9	–	–
Forebody as % BL	17–31	27	21	18[a]	17–25
Anterior testis length, as % BL	89–109, 10–14	86, 10	94, 10	90, 7	–
Anterior testis width, as % BW	109–123, 54–67	123, 60	118, 54	170, 46	–
Anterior testis position as % BL	60–64	62	61	52[a]	–
Posterior testis length, as % BL	95–104, 10–15	109, 12	100,11	130, 10	–
Posterior testis width, as % BW	97–110, 53–60	114, 56	112, 51	210, 57	–
Posterior testis position as % BL	70–73	73	68	62[a]	–
Post-testicular space as % BL	14–19	15	20	20[a]	–
Cirrus-sac length	120–133	128	141	173[a]	192[b]
Cirrus-sac width	60–69	61	57	62[a]	96[b]
Cirrus length as % BL	13–18	15	15	12	–
Cirrus-sac length to width ratio	1:0.50–0.55	1:0.48	1:0.40	1:0.36[a]	1:0.50[b]
Seminal vesicle length	116–142	142	129	–	204[b]
Seminal vesicle width	44–62	58	43	–	90[b]

Seminal vesicle as % length of cirrus-sac	96–113	111	91	–	106[b]
Genital pore as % BL	20–23	20	18	17[a]	–
Ovary length, as % BL	55–75, 6–8	71, 8	93, 10	90, 7	–
Ovary width, as % BW	79–97, 37–57	79, 39	97, 44	175, 47	–
Ovary position as % BL	54–61	58	58	46[a]	–
Postovarian space as % BL	34–37	34	33	46[a]	–
Egg length	71–74	69	77	160, 65[a]	64–85
Egg width	39–45	42	44	40, 37[a]	35–45
Number of eggs	4–7	10	17	14	–
Anterior extent of vitellarium as % BL	33–45	47	34	30	–
Number of vitelline follicles	95–126	112	107	–	–

aMeasurements derived from line drawing of Pearse (1924) using reported length of *P. boleosomi* as a scale
bMeasurements derived from line drawing of Pritchard (1966) using provided scale-bar
Abbreviations: BL, body length; BW, body width

Table 13 Pairwise comparisons of percent nucleotide similarity and the number of base pair differences (in parentheses) for the 28S, ITS-2, ITS-1 and 5.8S of species of *Plagioporus* provided in this study.

		Plagioporus limus n. sp.	*Plagioporus aliffi* n. sp.	*Plagioporus boleosomi*	*Plagioporus chiliticorum*	*Plagioporus hageli*	*Plagioporus kolipinskii*	*Plagioporus sinitsini*	*Plagioporus shawi*	*Plagioporus loboides*
28S	*Plagioporus fonti* n. sp.	97.4 (32)	97.9 (25)	99.3 (9)	97.0 (37)	97.3 (33)	96.3 (45)	97.2 (34)	96.1 (48)	97.9 (25)
	Plagioporus limus n. sp.	-	97.5 (30)	97.5 (31)	97.0 (36)	97.6 (29)	96.3 (45)	97.5 (30)	96.2 (46)	97.8 (27)
	Plagioporus aliffi n. sp.	-	-	97.9 (26)	96.9 (38)	97.5 (31)	96.3 (45)	97.5 (31)	96.1 (48)	97.9 (26)
ITS-2	*Plagioporus fonti* n. sp.	93.6 (16)	96.8 (8)	98.4 (4)	94.8 (13)	96.4 (9)	95.6 (11)	97.2 (7)	89.6 (25)	NA
	Plagioporus limus n. sp.	-	94.8 (13)	94.8 (13)	92.8 (18)	94.4 (14)	93.5 (16)	95.2 (12)	87.1 (31)	NA
	Plagioporus aliffi n. sp.	-	-	98.4 (4)	94.0 (15)	95.6 (11)	95.6 (11)	96.8 (8)	88.8 (27)	NA
ITS-1	*Plagioporus fonti* n. sp.	85.8 (91)	90.0 (66)	96.8 (21)	85.2 (97)	82.1 (117)	76.7 (153)	86.9 (83)	81.4 (91)	NA
	Plagioporus limus n. sp.	-	87.9 (78)	86.7 (85)	86.3 (86)	84.1 (101)	76.3 (152)	87.3 (78)	83.0 (78)	NA
	Plagioporus aliffi n. sp.	-	-	91.6 (55)	85.4 (95)	84.3 (103)	75.9 (160)	88.9 (71)	83.3 (80)	NA
5.8S	*Plagioporus fonti* n. sp.	99.4 (1)	99.4 (1)	100 (0)	98.7 (2)	99.4 (1)	98.7 (2)	99.4 (1)	98.7 (2)	NA
	Plagioporus limus n. sp.	-	100 (0)	99.4 (1)	99.4 (1)	100 (0)	99.4 (1)	99.4 (1)	98.7 (2)	NA
	Plagioporus aliffi n. sp.	-	-	99.4 (1)	99.4 (1)	100 (0)	99.4 (1)	99.4 (1)	98.7 (2)	NA

Table 14 Sequences retrieved from GenBank used for phylogenetic analysis.

Family	Species	Host	GenBank No.	Reference
Acanthocolpidae	*Stephanostomum pristis* (Deslongchamps, 1824)	*Phycis phycis* (Linnaeus)	DQ248222	Bray et al. (2005)
Brachycladiidae	*Zalophotrema hepaticum* Stunkard & Alvey, 1929	*Zalophus californianus* (Lesson)	AY222255	Olson et al. (2003)
Opecoelidae	*Allopodocotyle margolisi* Gibson, 1995	*Coryphaenoides mediterraneus* (Giglioli)	KU320596	Bray et al. (2016)
Opecoelidae	*Anomalotrema koiae* Gibson & Bray, 1984	*Sebastes viviparus* Krøyer	KU320595	Bray et al. (2016)
Opecoelidae	*Bathycreadium brayi* Pérez-del-Olmo, Dallarés, Carrassón & Kostadinova, 2014	*Trachyrincus scabrus* (Rafinesque)	JN085948	Constenla et al. (2011)
Opecoelidae	*Buticulotrema thermichthysi* Bray, Waeschenbach, Dyal, Littlewood & Morand, 2014	*Thermichthys hollisi* (Cohen, Rosenblatt & Moser)	KF733984	Bray et al. (2014)
Opecoelidae	*Dimerosaccus oncorhynchi* (Eguchi, 1931)	*Oncorhynchus masou* (Brevoort)	FR870252	Shedko et al. (2015)
Opecoelidae	*Gaevskajatrema halosauropsi* Bray & Campbell, 1996	*Halosauropsis macrochir* (Günther)	AY222207	Olson et al. (2003)
Opecoelidae	*Macvicaria mormyri* (Stossish, 1885)	Unidentified fish host	AF184256	Tkach et al. (2001)
Opecoelidae	*M. obovata* (Molin, 1859)	*Cyclope neritea* (Linnaeus)	JQ694147	Born-Torrijos et al. (2012)
Opecoelidae	*Neolebouria lanceolata* Andres, Pulis & Overstreet, 2014	*Polymixia lowei* (Günther)	KJ001210	Andres et al. (2014)
Opecoelidae	*Neoplagioporus ayu* (Takahashi, 1928)	*Plecoglossus altivelis altivelis* (Temminck & Schlegel)	KX553947	Fayton et al. (2016)
Opecoelidae	*Neoplagioporus elongatus* (Goto & Ozaki, 1930)	*Sarcocheilichthys variegatus microoculus* Mori	KX553948	Fayton et al. (2016)
Opecoelidae	*Neoplagioporus zacconis* (Yamaguti, 1934)	*Opsariichthys platypus* (Temminch & Schlegel)	KX553949	Fayton et al. (2016)

Opecoelidae	*Opecoeloides fimbriatus* (Linton, 1910)	*Micropogonias undulatus* (Linnaeus)	KJ001211	Andres et al. (2014)
Opecoelidae	*Plagioporus loboides* (Curran, Overstreet & Tkach, 2007)	*Fundulus nottii* (Agassiz)	EF523477	Curran et al. (2007)
Opecoelidae	*Plagioporus boleosomi* (Pearse, 1924)	*Percina maculata* (Girard)	KX553953	Fayton et al. (2016)
Opecoelidae	*Plagioporus carolini* Fayton, McAllister, & Connior 201X	*Cottus carolinae* (Gill)	NNXXXX	XXXXXXXXXX
Opecoelidae	*Plagioporus chiliticorum* (Barger & Esch, 1999)	*Notropis chiliticus* (Cope)	KX553943	Fayton et al. (2016)
Opecoelidae	*Plagioporus hageli* Fayton & Andres, 2016	*Oncorhynchus mykiss* (Walbaum)	KX553950	Fayton et al. (2016)
Opecoelidae	*Plagioporus icatluri* Fayton, McAllister, & Robison, 201X	*Noturus lachneri* Taylor	NNXXXX	XXXXXXXXXX
Opecoelidae	*Plagioporus kolipinskii* Tracey, Choudhury, Cheng & Ghosh, 2009	*Gasterosteus aculeatus* Linnaeus	KX553952	Fayton et al. (2016)
Opecoelidae	*Plagioporus shawi* (McIntosh, 1939)	*Oncorhynchus tshawytscha*	KX553951	Fayton et al. (2016)
Opecoelidae	*Plagioporus sinitsini* Mueller, 1934	*Notemigonus crysoleucas* (Mitchill)	KX553944	Fayton et al. (2016)
Opecoelidae	*Podocotyloides brevis* Andres & Overstreet, 2013	*Conger esculentus* Poey	KJ001212	Andres et al. (2014)
Opecoelidae	*Pseudopecoeloides tenuis* Yamaguti, 1940	*Priacanthus hamrur* (Forsskål)	KU320605	Bray et al. (2016)
Opecoelidae	*Urorchis acheiloghathi* Yamaguti, 1934	*Tanakia limbata* (Temminck & Schlegel)	KX553945	Fayton et al. (2016)
Opecoelidae	*U. goro* Ozaki, 1927	*Rhinogobius* sp.	KX553946	Fayton et al. (2016)

Figure 6. Plagioporus boleosomi from the intestine of *Percina caprodes*. 1, Ventral view; 2, Dorsal view; 3, Terminal genitalia, ventral view; 4, Female complex, dorsal view. Scale bars for 1-2: 100 µm, Scale bars for 3-4: 50 µm

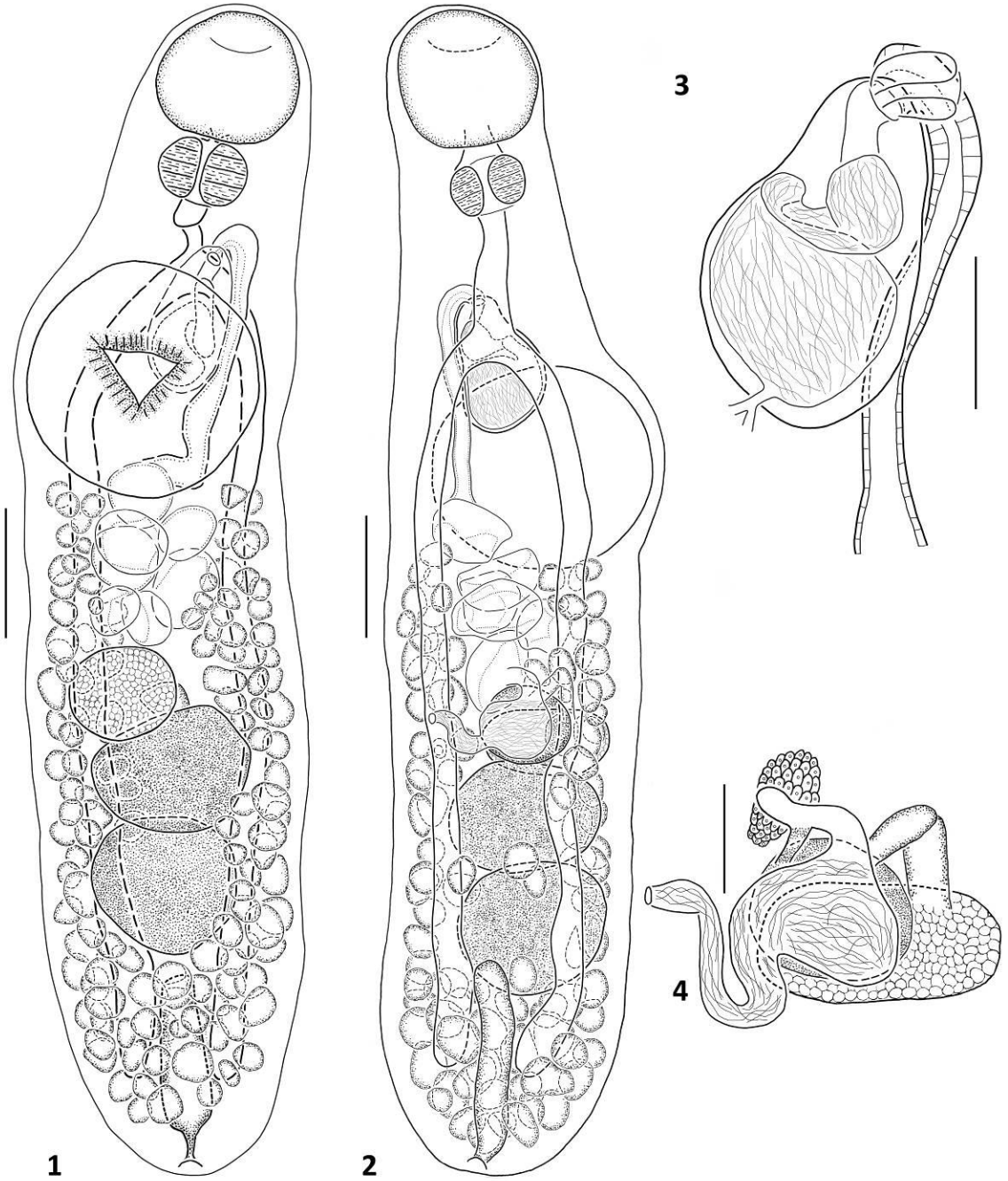


Figure 7. Plagioporus fonti n. sp. from the intestine of *Percina nigrofasciata*. 5, Ventral view; 6, Dorsal view; 7, Terminal genitalia, dorsal view; 8, Female complex, dorsal view. Scale bars for 5-6: 100 µm, Scale bars for 7-8: 50 µm

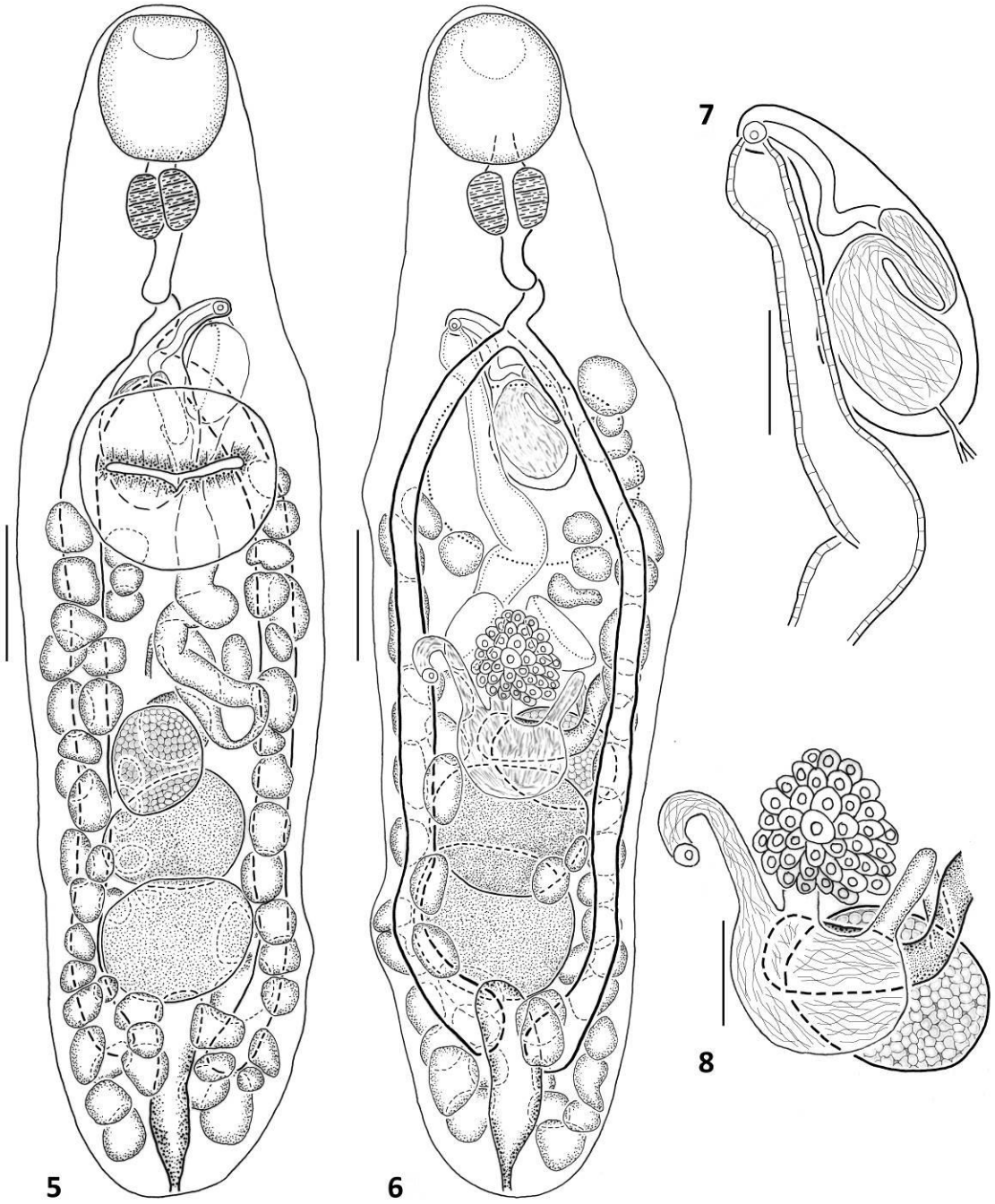


Figure 8. Plagioporus limus n. sp. from intestine of *Etheostoma squamosum*. 9, Ventral view; 10, Dorsal view; 11, Terminal genitalia, ventral view; 12, Female complex, dorsal view. Scale bars for 9-10: 100 μm, Scale bars for 11-12: 50 μm

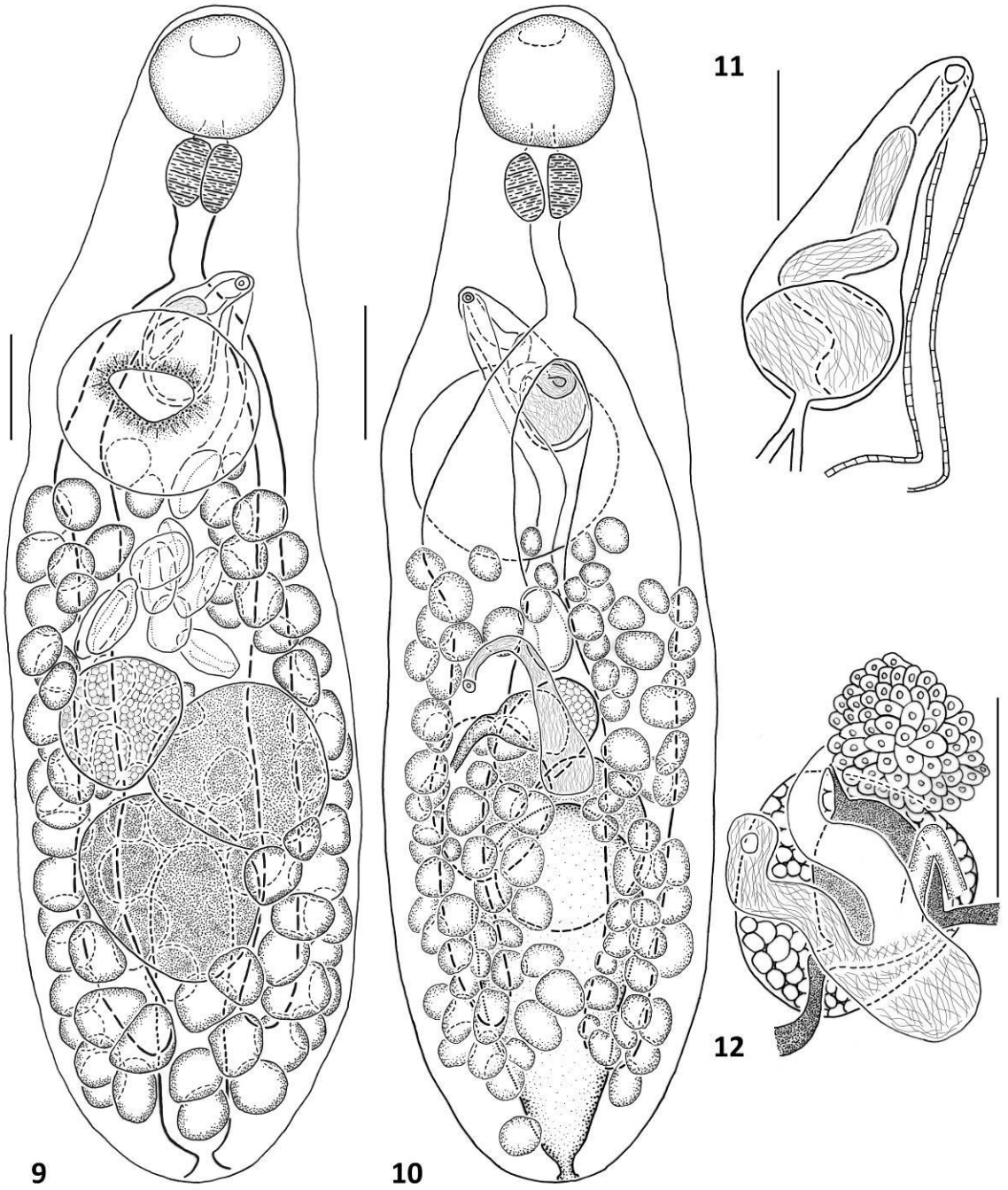


Figure 9. Plagioporus aliffi n. sp. from gut of *Etheostoma blennioides newmanni*. 13, Ventral view; 14, Dorsal view; 15, Terminal genitalia, ventral view; 16, Female complex, dorsal view. Scale bars for 13-14: 100 μm, Scale bars for 15-16: 50 μm

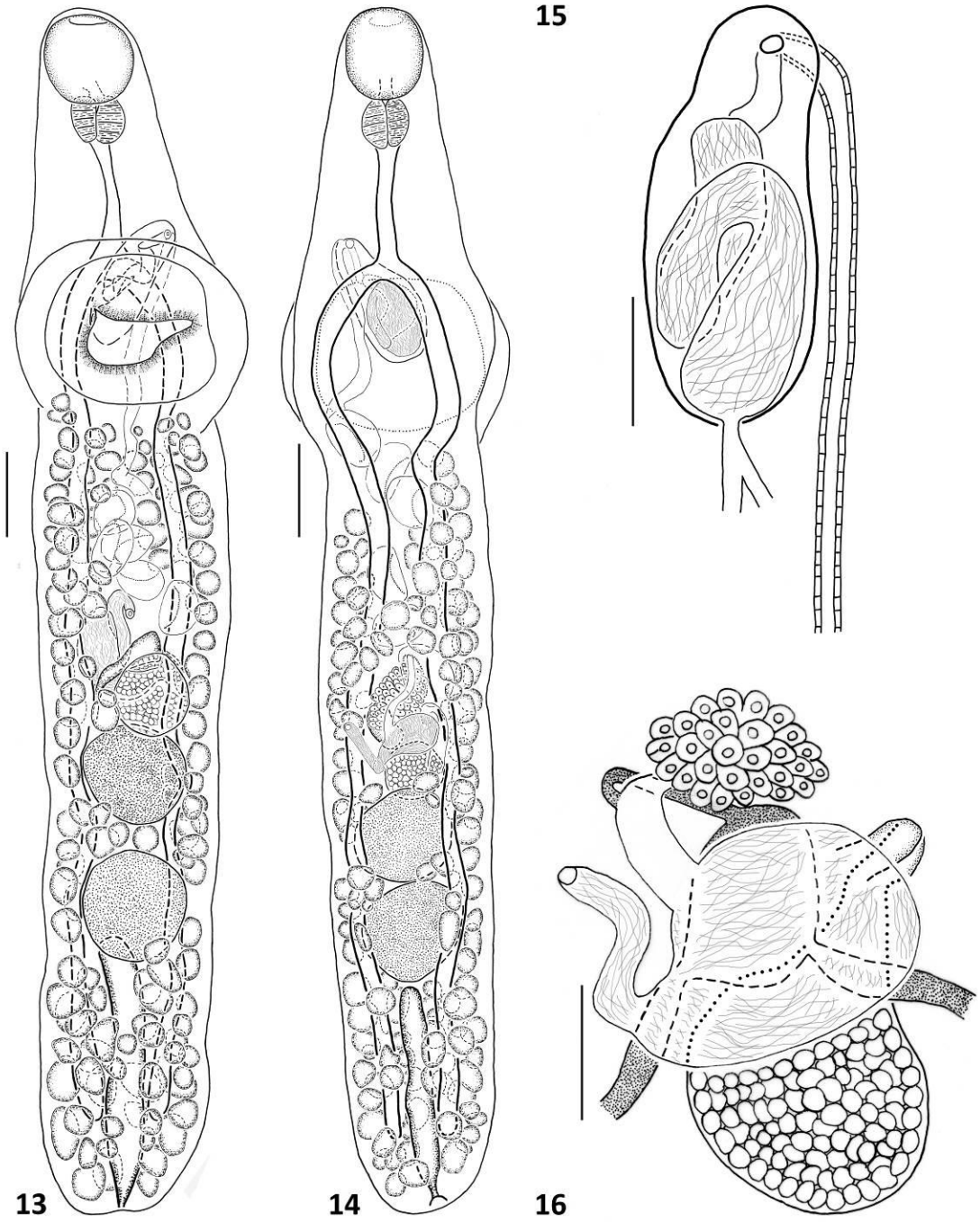
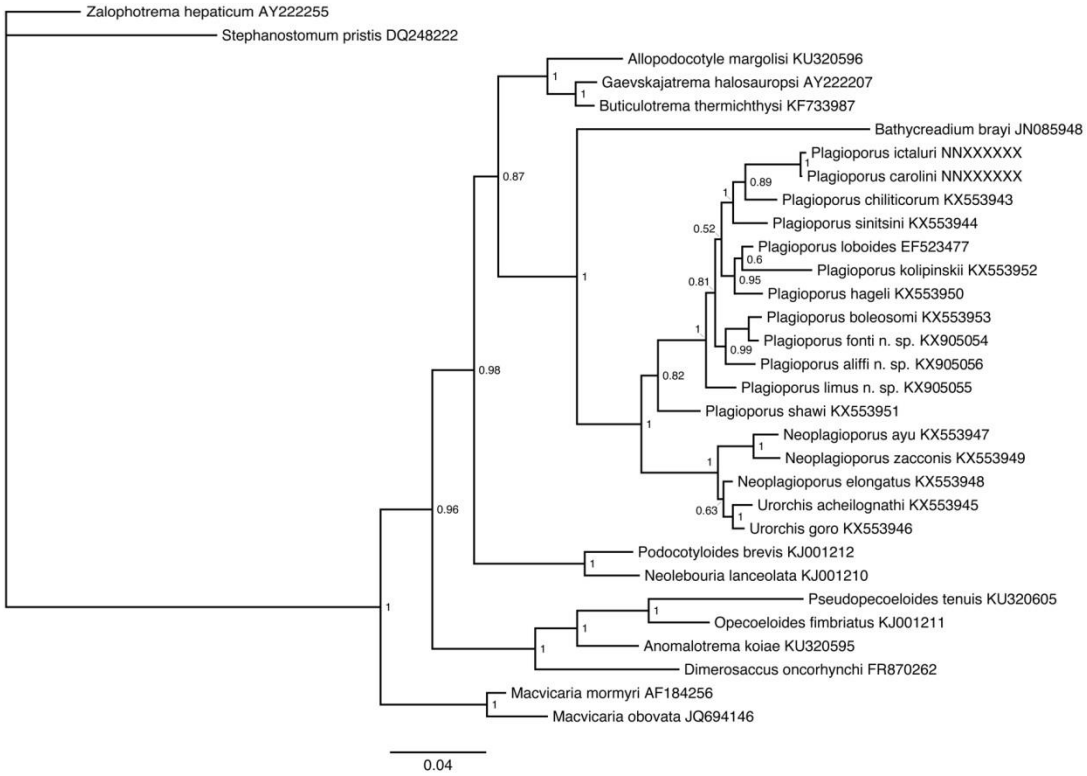


Figure 10. Phylogenetic relationships among members of *Plagioporus* resulting from Bayesian inference analysis of partial 28S rDNA sequences (GTR + I + Γ) (5,000,000 generations and a sample frequency of 1,000).



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CHAPTER V – Two new intestinal species of *Plagioporus* Stafford, 1904 of cyprinids from Tennessee and Virginia, with a redescription of *Plagioporus chiliticorum* (Barger & Esch, 1999) Cribb, 2005 from North Carolina

Abstract

Two new species of *Plagioporus* Stafford, 1904 are described from the intestine of cyprinids, including *Plagioporus crookedensis* n. sp. from *Clinostomus funduloides* Girard from Crooked Creek, Virginia, and *Plagioporus franksi* n. sp. from *Rhinichthys* spp. from Cosby Creek, Tennessee. *Plagioporus chiliticorum* (Barger & Esch, 1999) Cribb, 2005 is redescribed from *Notropis chiliticus* (Cope) from Basin Creek, North Carolina, its type locality and host. The 2 new species and *P. chilitcorum* are most similar to one another and are distinguished from congeners in possessing a combination of a circumcaecal vitellarium distributed in two distinct, lateral fields and a bipartite seminal vesicle. *Plagioporus franksi* n. sp. is most easily distinguished from the other new species and *P. chiliticorum* in having a more extensive vitelline field that extends anteriorly into the forebody. *Plagioporus crookedensis* n. sp. can be distinguished from *P. chiliticorum* in having a lower maximum anterior extent of the vitellarium at the posterior margin as opposed to the anterior margin of the ventral sucker. While most characters overlapped in range between *P. chiliticorum* and *Plagioporus crookedensis* n. sp., some characters only slightly overlapped in range and could collectively be used to distinguish the species, including number of vitelline follicles, extent of vitellarium relative to hindbody length, posttesticular space, and length and length to width ratio of the excretory bladder. A Bayesian inference (BI) analysis of partial 28S rDNA sequences was conducted using the 2 new species and 58 sequences of opecoelids obtained from GenBank and resolved

species of *Plagioporus* from the Nearctic with a vitellarium consisting of two distinct lateral fields and without a uterus extending to the end of the body as a highly supported clade within *Plagioporus*. *Plagioporus franksi* n. sp. was resolved as sister to *P. chiliticorum*, and in turn these species were sister to a clade containing *Plagioporus carolini* Fayton, McAllister & Connior, 2017 and *Plagioporus ictaluri* Fayton & Robison, 2017, a finding that adds support for a host switching event between cyprinids and cottids or ictalurids by intestinal *Plagioporus* spp. in the eastern Nearctic. *Plagioporus sinitsini* and in turn *Plagioporus crookedensis* n. sp. were sister to these 4 species with low and high support, respectively. The lack of a close relationship between *Plagioporus crookedensis* n. sp. and *P. chiliticorum* given their near cryptic morphological relationship, common parasitism of *C. funduloides*, and close biogeographic proximity emphasizes the importance of the inclusion of sequence data in taxonomic studies of opecoelids, a family rife with such homoplasies.

Introduction

Of the 18 valid species of *Plagioporus* Stafford, 1904 in the Nearctic, only 4 species parasitize the intestine of cyprinids. Three of these species have not been reported from outside of their type localities since their description and include *Plagioporus macrouterinus* Haderlie, 1953 from *Ptychocheilus grandis* (Ayres) from Deer Creek, California, *Plagioporus loboides* (Curran, Overstreet, & Tkach, 2007) Fayton & Andres, 2016 from *Notemigonus crysoleucas* (Mitchill) and *Fundulus* spp. from the Pascagoula River, Mississippi, and *Plagioporus chiliticorum* (Barger & Esch, 1999) Cribb, 2005 from *Notropis chiliticus* (Cope) from Basin Creek, North Carolina. *Plagioporus cooperi* (Hunter & Bangham, 1932) Price, 1934 was described from 7 species of cyprinids of

Lake Eerie, has since been reported mostly from cyprinid hosts from Kentucky, Wyoming and Michigan (Hoffman, 1999), and most recently was reported from *Notropis hudsonius* (Clinton) from the Richelieu River, Quebec (Marcogliese et al., 2016). In addition to these valid species, 2 *nomina nuda* species (sensu Tracey et al., 2009) of intestinal *Plagioporus* from cyprinids are known from the Nearctic, including Plagioporus elongatus from *Pimephales notatus* (Rafinesque) from Boone Creek, Kentucky, of Aliff (1973), and Plagioporus tennesseensis from *Campostoma anomalum* (Rafinesque) from Tennessee (only known from slides deposited in the NMNH by Leon Duobinis-Gray [Tracy et al., 2009]). Thus, undocumented diversity of intestinal *Plagioporus* may persist in the Nearctic

During a parasitological survey of freshwater fish in the eastern U.S.A., 2 morphologically distinct forms of *Plagioporus* were sampled from the intestine of cyprinids, including one from *Clinostomus funduloides* Girard from Crooked Creek outside of Galax, Virginia, and another from *Rhinichthys cataractae* (Valenciennes) and *Rhinichthys obtusus* Agassiz from Cosby Creek in the Great Smokey Mountain National Park, Tennessee. I provide a supplemental description of *P. chiliticorum* from its type host and locality using newly collected material and describe 2 new species of *Plagioporus* from the intestine of cyprinids using morphological and molecular methods, with the latter including the use ribosomal DNA sequence data to assess the phylogenetic relationships of the new species with *P. chiliticorum* and other congeners.

Material and Methods

On July 20th-21st, 2012 and later on August 17th-18th, 2013 specimens of *Plagioporus* herein considered to be a new species were harvested from the intestine of

C. funduloides from the East Fork of Crooked Creek outside of Galax, Virginia off of Pipers Gap Road (36° 38' 24.64"N, 80° 45' 51.25"W) collected via kicknet and bait trap. On July 30th, 2014 *R. obtusus* and *R. cataractae* infected with an additional form of Plagioporus were sampled via backpack electroshocker from Cosby Creek in the Great Smokey Mountains National Park, Tennessee (35° 46’ 39.94”N, 83° 12’ 46.75”W). On July 18th-19th, 2012 *Plagioporus chiliticorum* was harvested from *Notropis chiliticus* and *Clinostomus funduloides* (sampled via bait trap and kicknet) from Basin Creek, North Carolina off of Longbottom Road (36° 22’ 29.52”N, 81° 08’ 41.20”W). Specimens of opecoelids were excised from the intestine of fish hosts with the aid of a fine paintbrush and transferred to and observed in a shallow dish containing 0.6% saline. Subsequently, most of the saline was removed from this dish to the point where worms were subject to a vertically restricted plane of movement and suckered onto the glass, upon which near-boiling water was rapidly added to kill worms, minimizing curling post-fixation. Heat-killed worms were immediately transferred to 10% neutral phosphate buffered formalin for morphological examination or 95% ethanol for molecular analysis. Worms were stained in Mayer’s or Ehrlich’s haematoxylin or acetocarmine, dehydrated in a graded ethanol series, cleared in methyl salicylate and mounted permanently in Canada balsam or Damar gum. Helminth specimens collected during the present study were deposited in the United States National Parasite Collection at the Smithsonian National Museum of Natural History (NMNH), Washington, D.C. (Table 15). Specimens were examined using bright-field and differential interference contrast optics on an Olympus BX 51 microscope and illustrated using an attached drawing tube. Measurements are given in micrometers (µm) unless otherwise specified and are expressed as the measurements of

the holotype followed by the maximum and minimum values of paratypes in parentheses. For the supplemental description of *P. chiliticorum*, the average followed by the maximum and minimum values of vouchers is presented. The length and width of vitelline follicles are expressed as averages and standard deviations of 10 random follicles distributed throughout the body. Characters expressed as a measurement followed by body length (BL) refer to the distance from the anterior end.

Genomic DNA was isolated from each species of *Plagioporus* (number of replicates per species displayed in Table 15) using Qiagen DNAeasy Tissue Kit (Qiagen, Inc., Valencia, California, USA) following the instructions provided. DNA fragments c. 2,550 base pairs (bp) long, comprising the 3′ end of the 18S nuclear rDNA gene, internal transcribed spacer region (including ITS1 + 5.8S + ITS2) and the 5′ end of the 28S rDNA gene (including variable domains D1–D3), were amplified from the extracted DNA by polymerase chain reaction (PCR) on a PTC-200 Peltier Thermal Cycler using forward primer ITSF (5′ CGC CCG TCG CTA CTA CCG ATT G-3′) and reverse primer 1500R (5′ GCT ATC CTG AGG GAA ACT TCG-3′). These PCR primers and multiple internal primers were used in sequencing reactions. The internal forward primers were DIGL2 (5′ AAG CAT ATC ACT AAG CGG-3′), 300F (5′ CAA GTA CCG TGA GGG AAA GTT G-3′) and 900F (5′ CCG TCT TGA AAC ACG GAC CAA G-3′) and the internal reverse primers were 300R (5′ CAA CTT TCC CTC ACG GTA CTT G-3′), DIGL2R (5′ CCG CTT AGT GAT ATG CTT-3′) and ECD2 (5′ CTT GGT CCG TGT TTC AAG ACG GG-3′) (for primers see Littlewood et al., 2000; Tkach et al., 1999, 2000, 2001, 2003; Tkach & Snyder, 2007). The resulting PCR products were excised from PCR gels using QIAquick Gel Extraction Kit (Qiagen, Inc., Valencia, California, USA) following the

manufacturer's instructions, cycle-sequenced using ABI BigDyeTM chemistry (Applied Biosystems, Inc., Carlsbad, California, USA), ethanol-precipitated, and run on an ABI 3130 Genetic AnalyzerTM. The sequences of the 3 new species herein described were assembled using SequencherTM (GeneCodes Corp., Ann Arbor, Michigan, USA, Version 4.10.1) and submitted to Genbank (Table 15). The sequences were aligned using MAFFT version 6.611b (Katoh et al., 2005) with 1,000 cycles of iterative refinement and the genafpair algorithm. The boundaries between the 5.8S gene, ITS2 and 28S gene fragment were located using the ITS2 Ribosomal Database (Keller et al., 2009). Pairwise sequence comparisons of the complete ITS1, 5.8S and ITS2 and partial 28S nuclear rDNA genes of the 2 new species of *Plagioporus* from this study and available sequences of *Plagioporus* from GenBank were calculated with MEGA v6 using the "compute pairwise differences function," with gaps treated using the "pairwise deletion" function (Table 17). For phylogenetic analysis, sequences of opecoelids were obtained from GenBank (Table 16). The resulting alignment utilized 60 opecoelids, an acanthocolpid, a lepcreadiid and an enenterid and used the brachycladiid *Zalophotrema hepaticum* Stunkard & Alvey, 1929 as the outgroup based its phylogenetic position relative to the Opecoelidae (Olson et al., 2003) and to be comparable with the phylogeny presented by Bray et al. (2016). Phylogenetic analysis of the data was performed using BI with MrBayes 3.2.6 software (Huelsenbeck & Ronquist, 2001) run on the CIPRES portal (Miller et al., 2010) (Figure 12). The best nucleotide substitution model was estimated with jModeltest-2 (Darriba et al., 2012) as general time reversible with estimates of invariant sites and gamma-distributed among site-rate variation (GTR + I + Γ). The following model parameters were used in MrBayes: nst = 6, rates = invgamma, ngen =

5,000,000 and samplefreq = 1,000. Burn-in value was 5,000 estimated by plotting the log-probabilities against generation and visualizing plateau in parameter values (sump burnin = 5,000); nodal support was estimated by posterior probabilities (sumt) (Huelsenbeck et al., 2001) with all other settings left as default.

Redescription of *Plagioporus chiliticorum* (Barger & Esch, 1999) Cribb, 2005

Plagioporus chilitcorum (Barger & Esch, 1999) Cribb, 2005

Type-host: Redlip shiner, *Notropis chiliticus* (Cope) (Cypriniformes: Cyprinidae)

Other host: Rosyside dace, *Clinostomus funduloides* Girard (Cypriniformes: Cyprinidae)

Locality: Basin Creek, North Carolina (type locality) off of Longbottom Road (36° 22' 29.52"N, 81° 08' 41.20"W)

Site: Intestine

Prevalence: 5 of 20 *Notropis chiliticus* (25%)

Intensity: 1-4 per host

Type-material: Paratypes (USNM XXXXXXX-X).

Representative DNA sequences: Partial ITS1 and complete ITS2 regions, 5.8S gene, partial (D1–D3) 28S: GenBank accession no. NNXXXXXX, from 3 identical sequences.

Redescription (Figs11.1-3)

[Measurements based on 6 gravid wholemounts from *Notropis chiliticus*.] Body white to yellow in life, cylindrical to elongate cylindrical with bluntly rounded ends, 1,365 (979-2,057) long, 323 (246-427) wide, with maximum width in middle 50% BL. Tegument

smooth. Forebody 445 (288-585), representing 33 (28-42)% of body length (BL). Oral sucker subterminal to terminal, wider than long, 143 (102-203) long, 164 (135-214) wide. Ventral sucker sunken, wider than long, 200 (155-275) long, 217 (170-314) wide; width representing 66 (58-74)% of body width. Ratio of oral sucker to ventral sucker width 1:1.3 (1:1.2-1.5). Prepharynx 15 (8-19) long. Pharynx contiguous with to overlapping oral sucker by 1/2 of length, wider than long, 85 (70-118) long, 94 (75-121) wide. Esophagus 139 (118-164) long. Caecal bifurcation immediately anterior to ventral sucker to half of the distance between the anterior margin of the ventral sucker and posterior margin of the pharynx at 340 (249-463) BL, representing 26 (23-31)% of BL. Caeca extend posteriorly beyond posterior testis nearly to end of body, with or without turn before reaching level of vitellarium. Postcaecal space 105 (60-173), representing 8 (6-11)% of BL.

Testes two, tandem, overlapping to contiguous; anterior testis subequal, 116 (99-134) long, 111 (102-116) wide, with anterior extent at 917 (673-1418), representing 67 (62-69)% of BL; posterior testis subequal, overlapped by anterior testis by as much as 1/2 its length, 126 (112-139) long, 122 (107-134) wide, with anterior extent at 1,006 (748-1,515) BL, representing 74 (72-76)% of BL. Posttesticular space 236 (133-427), representing 17 (13-21)% of BL. Cirrus sac clavate, overlapping the ventral sucker in the anterior 1/4-3/4, 237 (187-332) long, 59 (47-70) wide, length representing 18 (13-22)% of BL. Internal seminal vesicle bipartite with chambers connected by distinct, thin duct, 139 (111-182) long, representing 60 (44-75)% length of cirrus sac, 45 (41-52) wide, occupying approximately 2/5-3/5 of sac, communicating with indistinct tubular region

(likely pars prostatica and ejaculatory duct). Gonopore ventrolateral, sinistral, in forebody at 331 (196-446), representing 24 (20-30)% of BL.

Ovary ovoid to triangular, dextral to median, slightly overlapping anterior testis to overlapping by 3/5 length, 103 (68-162) long, 98 (57-144) wide, with anterior margin at 873 (639-1,378) BL, representing 63 (56-67)% of BL. Oviduct extending posterodorsally from ovary, joining with canalicular seminal receptacle; seminal receptacle median, dorsal to anterior testis, extending slightly posterior to ovary. Laurer's canal extends anteriorly from seminal receptacle, opening submedian on dorsal surface anterior to ovary. Ootype extending anteriorly from seminal receptacle, joining Mehlis' gland at level of anterior margin of ovary. Uterus extending as far posteriorly level of posterior testis, with metraterm extending as far posteriorly as the posterior margin of the ventral sucker, containing 21 $\pm$ 19 (7-53) eggs. Eggs 71 (66-76) long, 39 (37-46) wide. Vitellarium follicular, in 2 lateral circumcaecal bunches, occasionally slightly overlapping in posttesticular space, anterior extent at 622 (384-935) BL, representing 45 (39-52)% of BL, with maximum anterior extent nearly to level of anterior margin of ventral sucker, posterior extent at 1,278 (950-1,902) BL, representing 94 (91-98)% of BL. Vitelline reservoir median, dorsal to anterior testis, ventral to seminal receptacle. Average length of 10 follicles 42 $\pm$ 11 (35-61) average width of 10 follicles 38 $\pm$ 11 (32-58). Vitellarium consisting of 190 (105-308) follicles. Common vitelline duct joining ootype anterior to Mehlis' gland.

Excretory vesicle tubular, with anterior extent posterior to posterior testis to overlapping it by 1/2 of length, 186 (134-266) long, representing 14 (12-15) % of BL; pore terminal.

Remarks

Gibson (1996) commented that the short excretory bladder of 2 of the 3 North American species from freshwater fish at that time maintained in the genus *Allopodocotyle* Pritchard, 1966 may require separation from the marine representatives of this genus. Cribb (2005) transferred these two species, *P. lepomis* Dobrovolny, 1939 and *P. boleosomi* (Pearse, 1924) Peters, 1957, along with *Plagioporus chilitcorum* (Barger & Esch 1999) Cribb, 2005 (originally placed in *Allopodocotyle* Pritchard, 1966 by Barger & Esch [1999]), to *Plagioporus*, noting that 'the possession of a short excretory bladder and parasitism of freshwater fishes probably indicates a relationship to *Plagioporus*.' *P. chiliticorum* was originally described from *Notropis chiliticus* and was later reported to also parasitize *C. funduloides* and *Semotilus atromaculatus* (Mitchill) from the type locality, Basin Creek, North Carolina (Barger & Esch, 2001). Barger and Esch (2001) examined 84 *Rhinicthys atratulus* (Hermann) and 68 *Oncorhynchus mykiss* (Walbaum) from the type locality over 5 months and found that these hosts were negative for *P. chiliticorum*. I dissected 20 *N. chilitcus*, 30 *C. funduloides* and 21 *Rhinicthys atratulus* (Hermann) from Basin Creek, North Carolina, and found that *P. chiliticorum* parasitized both *N. chiliticus* and *C. funduloides*.

The range of measurements of six gravid specimens of *Plagioporus* collected from *N. chiliticus* from Basin Creek, North Carolina overlap those reported for *P. chilitcorum* by Barger & Esch (1999) from the same host and locality, though my specimens had a lower minimum body length (body length of my specimens was 979-2,057 μm compared with 1,400-3,000 μm). Thus the minimum length and width for most characters is lower in my specimens, are I attribute this deviation to my inclusion of

specimens with body lengths below the minimum reported for *P. chiliticorum* by Barger and Esch (1999). Barger & Esch (1999) describe the vitellarium of *P. chiliticorum* as not confluent in the posttesticular space, though they noted the two fields encroached medially on the dorsal side. I report for the first time that the 2 lateral vitelline fields can slightly overlap (in my smallest specimen) in *P. chiliticorum*, but note that there is no distinct median field in the posttesticular space as in other congeners (the vitellarium is clearly associated with the caeca). Also, in 2 of my specimens the excretory bladder overlaps the posterior testis, extending no farther anteriorly than the middle of the posterior testis. Barger & Esch (1999) described the excretory vesicle of *P. chiliticorum* as never reaching the posterior testis. Barger & Esch (1999) also did not observe the degree of variation in the location of the maximum body width, which occurred in the middle 50 % of BL in my specimens (as opposed maximum body width at level of ventral sucker reported in original description). I also note that the seminal vesicle in my specimens overlaps the ovary, whereas Barger & Esch (1999) describe and illustrate the seminal vesicle as anterior to the ovary. In addition, there is a slight difference between my specimens and those of Barger and Esch (1999) in the range of the oral sucker to ventral sucker length ratios (1:1.3-1.6 in our specimens compared with 1:1.5-2.0). I consider all of these differences between my specimens and those of Barger & Esch (1999) to represent intraspecific variation for *P. chiliticorum*.

P. chiliticorum can be distinguished from *P. sinitsini* Mueller, 1934, *P. serotinus* Stafford, 1904, *P. cooperi* (Hunter & Bangham, 1932) Price, 1934, *P. boleosomi*, *P. hypentelii* Hendrix, 1973, *P. macrouterinus* Haderlie, 1953, *P. kolipinskii* Tracey, Choudhury, Cheng, & Ghosh, 2009, *P. siliculus* Sinitsin, 1931, *P. shawi* (McIntosh,

1939) Margolis, 1970, *P. loboides* (Curran, Overstreet & Tkach, 2007) Fayton & Andres, 2016, *P. hageli* Fayton & Andres, 2016, *P. carolini* Fayton, McAllister, & Connior, 2017, *P. ictaulri* Fayton, Robison & McAllister, 2017, *P. fonti* Fayton, Choudhury, McAllister, & Robison, 2017, *P. limus* Fayton, Choudhury, McAllister, & Robison, 2017, *P. aliffi* Fayton, Choudhury, McAllister, & Robison, 2017, P. elongatus and P. tennesseensis in possession of a bipartite seminal vesicle and from *P. lepomis* in having the vitellarium consitsting of two lateral, circumcaecal fields as opposed to having the vitellarium confluent in the posttesticular space and entirely ventral to the caeca. *P. chiliticorum* is morphologically most similar to *P. loboides*, *P. carolini*, *P. ictaluri*, *P. sinitsini*, and P. tennesseensis in having the vitellarium consisting of two distinct lateral fields. The vitellarium of *P. carolini* and *P. ictaluri* differs from that of *P. chiliticorum* in having sparse fields dorsal to the caeca (those of *P. chiliticorum* are circumcaecal) and having the anterior extent at the level of the pharynx as opposed to one not extending anteriorly beyond the anterior margin of the ventral sucker. *P. chiliticorum* further differs from *P. carolini* and *P. ictaluri* in having a longer posttesticular space (13-21% BL in *P. chiliticorum* as opposed to 4-7% BL in *P. ictaluri* and 1-6% BL in *P. carolini*), possession of caeca extending beyond the posterior testis (restricted posteriorly to the level of the posterior testis in *P. ictaluri* and *P. carolini*), generally having a shorter excretory bladder (12-15% BL in *P. chiliticorum* as opposed to 15-27% BL in *P. ictaluri* and 18-28% BL in *P. carolini*), and in parasitism of cyprinids (verses ictalurids for *P. ictaluri* and cottids and possibly ictalurids for *P. carolini*). *P. chiliticorum* can be further distinguished from *P. loboides* in having a uterus extending posteriorly only to the level of the posterior testis compared with one extending posteriorly to the end of the body and

in having a smaller postcaecal space (6-11 % BL as opposed to 14-25% in *P. loboides*) and further from *P. sinitsini* in having the vitellarium not distributed anteriorly beyond the anterior margin of the ventral sucker (distributed in forebody anteriorly at least to level of oesophagus in *P.* sinitsini) and in site of parasitism (intestine of cyprinids verses the gall bladder of cyprinids and catostomids in *P. sinitsini*). *P. chiliticorum* can be further distinguished from the *nomen nudum* species, P. tenneesseensis, in having an entire ovary as opposed to one with 3 to 4 lobes and in lacking vitellarium distributed in the forebody.

Description of *Plagioporus crookedensis* n. sp.

Plagioporus crookedensis n. sp.

Type and only known host: Rosyside dace, *Clinostomus funduloides* Girard (Cypriniformes: Cyprinidae)

Type Locality: East Fork of Crooked Creek outside of Galax, Virginia off of Pipers Gap Road (36° 38' 24.64"N, 80° 45' 51.25"W)

Site: Intestine

Prevalence: 8 of 12 *C. funduloides* (67%)

Intensity: 1-15 per host

Type-material: Paratypes (USNM XXXXXXX-X).

Representative DNA sequences: Partial ITS1 and complete ITS2 regions, 5.8S gene, partial (D1–D3) 28S: GenBank accession no. NNXXXXXX, from 3 identical sequences. East Fork of Crooked Creek outside of Galax, Virginia off of Pipers Gap Road (36° 38' 24.64"N, 80° 45' 51.25"W)

Etymology: The species is named after the type locality, Crooked Creek, from the east fork of which infected *C. funduloides* were collected.

Description (Figs. 12.4-7)

[Measurements based on 9 gravid wholemounts from *Clinostomus funduloides*.] Body white to yellow in life, cylindrical with bluntly rounded posterior end and narrowing in width approximately in anterior 1/4-1/3 of body, 3,172 (1,313-3,088) long, 619 (307-712) wide. Tegument smooth. Forebody 891 (363-919), representing 28 (23-36)% of body length (BL). Oral sucker subterminal to terminal, subequal, 328 (134-320) long, 325 (173-343) wide. Ventral sucker wider than long, 427 (213-408) long, 495 (228-480) wide; width representing 80 (65-80)% of body width. Ratio of oral sucker to ventral sucker width 1:1.5 (1:1.3-1.7). Prepharynx 18 (0-12) long. Pharynx contiguous with to overlapping oral sucker by 35 of length, subequal, 162 (99-152) long, 167 (92-181) wide. Oesophagus with or without turn, 273 (110-283) long. Caecal bifurcation approximately at level of anterior margin of ventral sucker to middle of distance separating pharynx and ventral sucker, at 723 (320-721) BL, representing 23 (21-25)% of BL. Caeca extend posteriorly beyond posterior testis, with or without turn before reaching level of vitellarium. Postcaecal space 351 (108-347), representing 11 (6-16)% of BL.

Testis two, tandem to oblique, overlapping to contiguous; anterior testis subequal, 255 (87-192) long, 238 (76-201) wide, with anterior extent at 2,042 (796-2100), representing 64 (61-68)% of BL; posterior testis subequal, overlapped by anterior testis by as much as 1/4 its length, 264 (97-302) long, 244 (83-239) wide, with anterior extent

at 2,208 (843-2,265) BL, representing 70 (64-75)% of BL. Posttesticular space 665 (308-520), representing 21 (17-25)% of BL. Cirrus sac clavate, overlapping the ventral sucker in the anterior 1/5-3/5, 386 (180-361) long, 119 (50-117) wide, length representing 12 (12-15)% of BL. Internal seminal vesicle bipartite with chambers connected by distinct, thin duct, 237 (104-236) long, representing 61 (46-78)% length of cirrus sac, 105 (32-71) wide, occupying approximately 2/5-3/5 of sac, communicating with indistinct tubular region (likely pars prostatica and ejaculatory duct). Gonopore ventrolateral, sinistral, in forebody at 699 (277-717), representing 22 (20-25)% of BL.

Ovary ovoid, subequal, dextral to median, parallel to anterior testis or overlapping it by ½ its length, overlapping up to the anterior ¼ of the posterior testis, 252 (84-232) long, 181 (84-200) wide, with anterior margin at 1,897 (772-2,007) BL, representing 60 (55-65)% of BL. Oviduct extending anterodorsally from anterior ovary, turning posteriorly to join with canalicular seminal receptacle; seminal receptacle submedian, dorsal to anterior testis and ovary, extending posteriorly at most to level of the ovary. Laurer's canal extends anteriorly from seminal receptacle, opening submedian to sinistral on dorsal surface approximately halfway between the posterior margin of the ventral sucker and anterior margin of the ovary. Ootype extending anteriorly from seminal receptacle. Mehlis' gland conspicuous. Uterus dorsal and occasionally ventral to ovary, ventral to testis, extending as far posteriorly as midpoint of posterior testis, with metraterm at posterior margin of cirrus sac to anterior 1/3 of ventral sucker length, containing 4 to over 100 eggs. Eggs 81 (76 $\pm$ 7, 65-85) long, 37 (40 $\pm$ 6, 33-54) wide. Vitellarium follicular, in 2 lateral circumcaecal bunches, occasionally slightly overlapping to confluent in posttesticular space, maximum anterior extent slightly

anterior to posterior margin of ventral sucker, 1,445 (631-1,500) BL, representing 46 (41-60)% of BL, posterior extent at 2,925 (1,155-2.886) BL, representing 92 (83-97)% of BL. Vitelline reservoir median, dorsal to anterior testis, ventral to seminal receptacle. Average length of 10 follicles 72 (45 ± 17, 22-62) average width of 10 follicles 67 (40 ± 16, 20-55). Vitellarium consisting of 275 (272-358) follicles. Common vitelline duct joining ootype at level of anterior margin of ovary.

Excretory vesicle tubular, extending anteriorly from 7/10 of the distance between the posterior margin of the posterior testis to slightly posterior to the posterior testis, never overlapping posterior testis, 516 (228-497) long, representing 16 (14-19)% of BL; pore terminal.

Remarks

Plagioporus crookedensis n. sp. can be distinguished from *P. sinitsini*, *P. serotinus*, *P. cooperi*, *P. boleosomi*, *P. hypentelii*, *P. macrouterinus*, *P. kolipinskii*, *P. siliculus*, *P. shawi*, *P. loboides*, *P. hageli*, *P. carolini*, *P. ictaulri*, *P. fonti*, *P. limus*, *P. aliffi*, P. elongatus and P. tenneesseensis in possession of a bipartite seminal vesicle. The new species is distinguished from *P. lepomis* in having the vitellarium consitsting of two circumcaecal fields as opposed to fields only ventral to the caeca that are confluent, forming a distinct median vitelline field, in the postesticular space. *Plagioporus crookedensis* n. sp. differs from *P. chiliticorum* in the distribution of the vitellarium; the maximum anterior extent of the vitellarium for *P. chilitcorum* is the anterior margin of the ventral sucker (the anterior extent of vitellarium was at the level of ventral sucker in half of the specimens of *P. chilitcorum* examined) as opposed one not reaching anteriorly beyond the posterior margin of the ventral sucker in the new species and in the

development of the vitellarium. When only considering specimens of *P. chiliticorum* in the lower 2/3 of examined body lengths, *Plagioporus crookedensis* n. sp. possesses a higher number of vitelline follicles across body lengths than *P. chiliticorum* [respectively 272-358 and 105-227 follicles]); the number of follicles is constant across body lengths in the new species whereas it seems to increase with increasing body length in *P. chiliticorum*. The new species is somewhat similar to *P. sinitsini*, *P. ictaluri*, *P. carolini*, *P. loboides* and P. tennesseensis in having lateral vitelline fields that are ventral and dorsal to the caeca. *Plagioporus crookedensis* n. sp. further differs from *P. carolini* and *P. ictaluri* in having a longer posttesticular space (17-25% BL in new species opposed to 4-7% BL in *P. ictaluri* and 1-6% BL in *P. carolini*), possession of caeca extending beyond the posterior testis as opposed to those restricted posteriorly to the level of the posterior testis) and in parasitism of cyprinids (verses ictalurids for *P. ictaluri* and cottids and possibly ictalurids for *P. carolini*). The new species can be further distinguished from *P. loboides* in having a uterus with a maximum posterior extent in the anterior half of the posterior testis compared with one extending posteriorly to the end of the body and in possession of vitellarium that does not extend anteriorly beyond the posterior margin of the ventral sucker (usually extends to middle of ventral sucker in *P. loboides*); from *P. sinitsini* in having the vitellarium restricted to the hindbody (distributed in forebody anteriorly at least to level of oesophagus in *P. sinitsini*) and in site of parasitism (intestine of cyprinids verses the gall bladder of cyprinids and catostomids in *P. sinitsini*); and from P. tenneesseensis in having an entire ovary as opposed to one with 3 to 4 lobes. *Plagioporus crookedensis* n. sp. is most similar to *P. chiliticorum* in possession of a bipartite seminal vesicle, vitelllarium consisting of 2 lateral, circumcaecal fields, a wider

than long ventral sucker and parasitism of *Clinostomus funduloides* in an Appalachian stream. While the ranges of most characters overlap between the new species and *P. chilitcorum*, the two species respectively diverge further as follows: testis oblique to tandem as opposed to only tandem; anterior testis can be parallel, tandem or dextral to ovary as opposed to only tandem to dextral to ovary; maximum posterior extent of uterus middle of posterior testis as opposed to one at the level of the anterior margin of the posterior testis, excretory vesicle extends anteriorly from the end of the body approximately 7/10 of the distance to the posterior testis to slightly posterior to the posterior testis (length represents 14-19 % of BL) as opposed to one posterior to overlapping the posterior testis with a length representing 12-15 % BL. Compared to *P. chiliticorum*, the new species tends to have a more restricted vitelline field (48-67 % as opposed to 58-88 % length of hindbody with respective average length of vitellarium 57 $\pm$ 6 % verses 74 $\pm$ 12 % of hindbody length), a longer posttesticular space (24-36 % compared with 19-26 % of hindbody length), a shorter esophagus (22-38 % verses 23-47 % length of forebody), a more tubular excretory vesicle (length to width ratio of excretory vesicle 1:0.6-0.20 as opposed to 1:0.16-0.41), a more restricted position of the genital pore in the forebody (70-89 % compared with 34-82 % length of forebody) and a greater body width. Bager & Esch [1999] report a maximum body width of 380-600 μm for *P. chiliticorum* whereas 30% of *Plagioporus crookedensis* n. sp. had body widths in excess of 600 μm up to 712 μm. In addition, the new species has a longer minimum body length of mature specimens compared with *P. chilitcorum*. Each of the infected *Clinostomus funduloides* infected with *Plagioporus crookedensis* n. sp. harbored mature and immature worms, and mature specimens were all at or above 1,313 μm in body

length (minimum size of maturity for *P. chilitcorum* is 979 μm). Notably, *Plagioporus crookedensis* n. sp. were not found in *Semotilus atromaculatus* (n=50) or *Rhinichthys atratulus* (n=15) collected from the type locality, though *S. atromaculatus* harbored mature specimens of *Allocreadium lobatum* Wallin, 1909.

Description of *Plagioporus franksi* n. sp.

Plagioporus franksi n. sp.

Type host: Longnose dace, *Rhinichthys cataractae* (Valenciennes) (Cypriniformes: Cyprinidae)

Other host: Western blacknose dace, *Rhinichthys obtusus* Agassiz (Cypriniformes: Cyprinidae)

Type Locality: Cosby Creek in the Great Smokey Mountains National Park, Tennessee (35° 46’ 39.94”N, 83° 12’ 46.75”W)

Site: Intestine

Prevalence: 4 of 4 R. cataractae (100%)

Intensity: 1-18 per host

Type-material: Paratypes (USNM XXXXXXX-X).

Representative DNA sequences: Partial ITS1 and complete ITS2 regions, 5.8S gene, partial (D1–D3) 28S: GenBank accession no. NNXXXXXX, from 3 identical sequences.

Etymology: The species is named after James S. Franks (Gulf Coast Research Laboratory, University of Southern Mississippi), a native of Newport, Tennessee, near the type locality, in recognition of his past and continuing research on pelagic and coastal fishes of the Gulf of Mexico.

Description (Figs. 13.8-11)

[Measurements based on 10 gravid wholemounts from *Rhinichthys cataractae*.] Body white to yellow in life, fusiform to cylindrical with bluntly rounded posterior end and narrowing in width approximately in anterior 1/3 of body, 903 (502-877) long, 232 (127-231) wide. Tegument smooth. Forebody 305 (158-265), representing 34 (30-35)% of body length (BL). Oral sucker subterminal, subequal, 113 (87-125) long, 123 (79-126) wide. Ventral sucker subequal, 148 (100-153) long, 163 (104-158) wide; width representing 70 (74 $\pm$ 5, 66-82)% of body width. Ratio of oral sucker to ventral sucker width 1:1.3 (1:1.2-1.4). Prepharynx 12 (7-17) long. Pharynx contiguous with to overlapping oral sucker by 1/3 of length, subequal, 61 (37-63) long, 73 (35-70) wide. Oesophagus with or without turn, 97 (56-107) long. Caecal bifurcation slightly anterior to ventral sucker to middle of distance separating pharynx and ventral sucker, at 244 (136-223) BL, representing 27 (22-30)% of BL. Caeca extend posteriorly beyond posterior testis. Postcaecal space 91 (25-99), representing 10 (4-11)% of BL.

Testes two, tandem to slightly oblique, overlapping to contiguous; anterior testis subequal, 120 (60-131) long, 101 (66-110) wide, with anterior extent at 570 (329-571), representing 63 (61-70)% of BL; posterior testis longer than wide, overlapped by anterior testis by as much as 1/3 of its length to contiguous with it, 146 (67-161) long, 105 (59-134) wide, with anterior extent at 650 (383-643) BL, representing 72 (70-79)% of BL. Posttesticular space 116 (45-126), representing 13 (7-16)% of BL. Cirrus sac clavate, overlapping the ventral sucker in the anterior 1/3-3/4, 192 (111-185) long, 60 (32-60)

wide, length representing 21 (18-27)% of BL. Internal seminal vesicle bipartite with chambers connected by distinct duct, 143 (78-140) long, representing 74 (65-91)% length of cirrus sac, 48 (25-47) wide, occupying approximately 1/2-2/3 of sac, communicating with indistinct tubular region (likely pars prostatica and ejaculatory duct). Gonopore ventrolateral, sinistral, in forebody at 214 (130-190), representing 24 (22-27)% of BL. Ovary ovoid, subequal, tandem to dextral to anterior testis, overlapping anterior testis by all to ¼ of length, 96 (39-106) long, 83 (36-115) wide, with anterior margin at 536 (315-515) BL, representing 59 (59-67)% of BL. Oviduct extending anterodorsally from anterior ovary, turning posteriorly to join with canalicular seminal receptacle; seminal receptacle median, dorsal to anterior testis and ovary, extending posteriorly to level of the ovary. Laurer's canal extends anteriorly from seminal receptacle, opening submedian to sinistral on dorsal surface approximately halfway between the posterior margin of the ventral sucker and anterior margin of the ovary. Ootype extending anteriorly from seminal receptacle. Mehlis' gland conspicuous. Uterus dorsal to ovary, ventral to testis, extending as far posteriorly as midpoint of anterior testis, with metraterm at posterior margin of cirrus sac to anterior 1/3 of ventral sucker length, containing 9 (2-16) eggs. Eggs 66 (52-73) long, 36 (35-44) wide. Vitellarium follicular, in 2 lateral bunches ventral and dorsal to the caeca, anterior margin at level of of caecal bifurcation, 229 (127-215) BL, representing 25 (22-30)% of BL, posterior extent at 843 (468-855) BL, representing 93 (96 $\pm$ 2, 93-98)% of BL. Vitelline reservoir median, dorsal to anterior testis, ventral to seminal receptacle. Average length of 10 follicles 34 (26 $\pm$ 10, 14-45) average width of 10 follicles 31 (22 $\pm$ 7, 14-35). Vitellarium consisting of 118 (98-152) follicles. Common vitelline duct joining ootype at level of anterior margin of ovary.

Excretory vesicle tubular, extending anteriorly to dorsally overlap the posterior testis by as much as ½ its length, 117 (67-159) long, representing 13 (11-19)% of BL; pore terminal.

Remarks

Plagioporus franksi n. sp. can be distinguished from *P. sinitsini*, *P. serotinus*, *P. cooperi*, *P. boleosomi*, *P. hypentelii*, *P. macrouterinus*, *P. kolipinskii*, *P. siliculus*, *P. shawi*, *P. loboides*, *P. hageli*, *P. carolini*, *P. ictaulri*, *P. fonti*, *P. limus* and *P. aliffi* in possession of a bipartite seminal vesicle. The new species is distinguished from *P. lepomis* in having the vitellarium consitsting of two circumcaecal fields as opposed to fields only ventral to the caeca that are confluent, forming a distinct median vitelline field, in the postesticular space and from *P. chiliticorum* and *P. crookedensis* in having the vitellarium extending anteriorly into the forebody. The new species is somewhat similar to *P. sinitsini*, *P. ictaluri*, *P. carolini*, *P. loboides* and P. tenneeseensis in having 2 lateral vitelline fields that are ventral and dorsal to the caeca but can be distinguished from these species respectively as follows: from *P. sinitsini* in having the anterior margin of the vitellarium at the level of the caecal bifurcation as opposed to one at the level of the pharynx and in site of parasitism (intestine verses gall bladder); from *P. ictaluri* and *P. carolini* in having the caeca terminate posterior to the posterior testis compared with a termination anterior to the midpoint of the posterior testis and in parasitism of cyprinids (verses ictalurids for *P. ictaluri* and cottids and possibly ictalurids for *P. carolini*); from *P. loboides* in having a uterus with a maximum posterior extent at the midpoint of the anterior testis compared with one extending posteriorly to the end of the body and in having a shorter postcaecal speace (4-11% BL as opposed to 14-25% BL), and from P.

tennesseensis in having an entire ovary as opposed to one with 3 to 4 lobes. *Plagioporus franksi* n. sp. is morphologically most similar to *P. chilitcorum* and *P. crookedensis* in having 2 lateral, circumcaecal vitelline fields, a bipartite seminal vesicle, and in parasitism of cyprinids. The new species can be further distinguished from these congeners in having a more extensive distribution of the vitellarium (66-76% BL as opposed to 39-58% BL and 33-49 % BL in *P. chiliticorum* and *P. crookedensis*, respectively), a smaller body length (502-903 μm compared with 1,313-3,088 μm and 979-2,057 μm in *P. chiliticorum* and *P. crookedensis*, respectively), a uterus that does not extend posteriorly beyond the midpoint of the anterior testis (extends to level of posterior testis in *P. chilitcorum* and *P. crookedensis*), and in parasitism of *Rhinichthys* spp. (as opposed to various other cyprinids for *P. chiliticorum* and *C. funduloides* for *P. crookedensis*). The number of vitelline follciles overlapped in *Plagioporus franksi* n. sp. (98-152 follicles) and *P. chiliticorum* (105-30 follicles), whereas *Plagioporus crookedensis* n. sp. had significantly more follicles than *Plagioporus franksi* n. sp. (272-358 follicles). Juvenile *Hypentelium nigricans* (Lesueur) (n=5) also collected from Cosby Creek, Tennessee, were negative for *Plagioporus franksi* n. sp.

Molecular Analysis

No intraspecific variation was observed for *Plagioporus crookedensis* n. sp. and *Plagioporus franksi* n. sp. (Table 15). Sequence lengths of the partial ITS1 rDNA gene used for pairwise comparisons for *P. boleosomi*, *P. fonti*, *P. limus*, *P. aliffi*, *P. chiliticorum*, *P. hageli*, *P. kolipinskii*, *P. sinitsini*, *P. shawi*, *P. carolini*, *P. ictaluri*, *Plagioporus crookedensis* n. sp. and *Plagioporus franksi* n. sp. were 613 bp, 620 bp, 625 bp, 623 bp, 615 bp, 811 bp, 661 bp, 600 bp, 451 bp, 900 bp, 867 bp, 618 bp and 615 bp

respectively. The length of the complete 5.8SrDNA gene and for all of these species was 156 bp and lengths of the partial 28S rDNA gene ranged from 1,196-1,199 bp. The length of the complete ITS2 rDNA gene was 250 bp for all species of *Plagioporus* examined except for those of *Plagioporus crookedensis* n. sp., *P. shawi*, *P. kolipinskii* and *P. aliffi*, which had respective lengths of 254, 240 bp, 251 bp, and 251 bp.

In the partial 28S rDNA gene, *Plagioporus crookedensis* n. sp. was most similar to *P. franksi* n. sp., *P. sinitsini* and *P. loboides* with a similarity of 98% for all three species; the next most similar species was *P. chiliticorum* (97.9 % similar). In the complete ITS2 rDNA gene, *Plagioporus crookedensis* n. sp. was most similar to *P. sinitsini* (98.4% similar), followed by *P. hageli*, *P. carolini*, *P. ictaluri* and *Plagioporus franksi* n. sp. with respective similarities of 97.6%, 96.8%, 96.4% and 96.4%. In the partial ITS1 rDNA gene, *Plagioporus crookedensis* was most similar to *P. sinitsini*, *P. chilitcorum* and *Plagioporus franksi* n. sp. with respective similarities of 92.8%, 90.9%, and 90.5%.

In the partial 28S rDNA gene, *Plagioporus franksi* n. sp. was most similar to *P. chiliticorum* (99.9% similar), followed by *Plagioporus crookedensis* n. sp., *P. sinitsini* and *P. loboides*, with respective similarities of 98.0%, 97.6% and 97.6%. *Plagioporus franksi* n. sp. was most similar to *P. chiliticorum* (99.6% similar) in the complete ITS2 rDNA gene, followed by *P. sinitsini* and *P. hageli* with a similarity for both of 96.4%. In the partial ITS1 gene, *Plagioporus franksi* n. sp. was again most similar to *P. chilitcorum* (99.7% similar), followed by *Plagioporus crookedensis* n. sp. and *P. sinitsini*, with respective similarities of 90.5% and 88.6%. Similarity in the complete 5.8S rDNA gene

ranged from 98.7-100% in both *Plagioporus franksi* n. sp. and *Plagioporus crookedensis* n. sp.

The alignment of the partial 28S rDNA gene sequences of the 2 new species and those of other opecoelids from GenBank was 1,360 bp with 728 conserved, 618 variable and 465 informative sites. *Plagioporus franksi* n. sp. was resolved as sister to *P. chiliticorum* and in turn these 2 species were the sister of a clade formed by *P. carolini* and *P. ictaluri*, with all relationships being resolved with high support. These 4 species of *Plagioporus* were sister to *P. sinitsini* and in turn to *Plagioporus crookedensis* n. sp., with low and high support, respectively. These 6 species were sister to a clade containing *P. loboides*, *P. hageli* and *P. kolipinskii* and in turn to a clade of *Plagioporus* species parasitizing darters (*P. boleosomi*, *P. fonti*, and *P. aliffi*), all with high support. *P. limas* and in turn *P. shawi* were resolved as sister to all other species of *Plagioporus* with high and low support, respectively. The topology of the BI analysis is consistent with that of Bray et al. (2016), though my analysis resolved several relationships that were resolved as polytomies by these authors. With respect to the BI analysis from chapter 2, this analysis is identical with the exception that *Gaevskajatrema perezi* (Mathias, 1926) was resolved as sister to a clade formed by *Propycnadenoides philippinensis* Fischthal and Kutz, 1964 and *Percreadium idoneum* (Nicoll, 1909), whereas in chapter 2 the latter species was resolved a sister to the 2 former species. Bray et al. (2016) resolved the relationship between these 3 species as a polytomy.

Discussion

No single morphological character definitively distinguished *Plagioporus crookedensis* n. sp. from *P. chiliticorum*, both of which parasitize cyprinids in

Appalachian streams separated by *c*. 45 km. However, many characters only slightly overlapped in range and can collectively be used to distinguish the 2 species, including the anterior extent of the vitellarium, number of vitelline follicles, extent of vitellarium relative to hindbody length, posttesticular space, and length and length to width ratio of the excretory bladder. *Plagioporus crookedensis* n. sp. and *P. chiliticorum* were the morphologically most similar congeners to *Plagioporus franksi* n. sp., and these 3 species are distinct from other Nearctic *Plagioporus* spp. in having a combination of a bipartite seminal vesicle and vitellarium consisting of 2 distinct lateral, circumcaecal fields.

My BI analysis resolved species of *Plagioporus* with distinct lateral vitelline fields without a uterus extending to the posterior end of the body, including the 2 new species, *P. sinitsini*, *P. ictaluri* and *P. carolini*, in a well supported clade within *Plagioporus* (Figure 14). The close relationship of *Plagioporus franksi* n. sp. to *P. chiliticorum* and *Plagioporus crookedensis* to *P. sinitsini* was corroborated by base pair comparisons of the partial ITS1 and 28S and complete ITS2 rDNA genes. Despite their common parasitism of *C. funduloides*, close biogeographic proximity, and near cryptic morphological relationship, *Plagioporus crookedensis* n. sp. and *P. chiliticorum* were more closely related to other species than they were to each other. This finding emphasizes the importance of the inclusion of sequence data and awareness of the possibility of cryptic species in taxonomic studies of opecoelids. Interestingly, despite the co-occurrence of the two new species in the Ohio river drainage, *Plagioporus franksi* n. sp. was not as closely related to *P. crookedensis* n. sp. as it was to *P. chiliticorum*, which occurs in an Atlantic drainage (Pee Dee River drainage). The low degree of divergence between *Plagioporus franksi* n. sp. and *P. chiliticorum* in the examined rDNA genes may

indicate that these are sister species; such a low divergence was also observed between the sister species *P. carolini* and *P. ictaluri* in Chapter 3. The biogeographic diversification of *Plagioporus* in the Nearctic is far from clear, and the nesting of species parasitizing cottids and ictalurids (*P. carolini* and *P. ictaluri*) within a clade primarily composed of species specific to cyprinids may indicate a host switching event across these fish families. In addition, the nesting of *P. sinitsini*, which is specific to the gall bladder of cyprinids and catostomids, within a derived clade of intestinal species indicates a switch from parasitism of the intestine to the gall bladder at some point in the evolutionary history of *Plagioporus*, though I note that this relationship was resolved with poor support. Sequencing of additional gall bladder forms and other intestinal species with distinct lateral vitelline fields (like P. tennesseensis) may clarify the biogeography and host associations involved in the shift to parasitism of the gall bladder in *Plagioporus*.

Plagioporus loboides was the only Nearctic species of *Plagioporus* with two distinct lateral vitelline fields included in my BI analysis that was not resolved in the clade with the 2 new species, *P. chiliticorum*, *P. sinitsini*, *P. carolini* and *P. ictaluri*; instead, it was resolved in a clade sister to the one formed by these species along with 2 species from the western Nearctic from secondary freshwater fishes. While P. *loboides* is most closely related to *P. hageli* (98.6% similar in 28S rDNA gene), the second most similar species to it is *Plagioporus crookedensis* n. sp. (98.0 % similar in 28S rDNA gene). Moreover, *P. loboides* was among the most similar congeners to the two new species in the 28S rDNA gene. Thus, given that the relationship of *P. loboides* was resolved with low support and its parasitism of cyprinids in the eastern Nearctic, it is

possible that such a restriction of the vitellarium is not an instance of homoplasy in *Plagioporus*, especially considering the possibly of undersampling of similar congeners. The inclusion of *Plagiocirrus* spp., which possess a uterus extending to the end of the body as in *P. loboides*, in future molecular phylogenies may clarify the evolution of this morphotype. The concurrent of possession vitellline fields restricted to two lateral fields and a posteriorly extending uterus does not seem to be a novel character for *Plagioporus* given the vitellarium distribution and maximum posterior extent of the uterus in *P. chiliticorum* and *Plagioporus crookedensis* n. sp, reinforcing the transfering of *P. loboides* to *Plagioporus* and generic amendments to accommodate it made in the second chapter.

Table 15 Species of *Plagioporus* collected from the Nearctic and their respective hosts, collection localities, GenBank accession number (with number of replicates in parenthesis) and deposition information.

Species	Host	Collection Locality and Date	GenBank	NMNH
Plagioporus chiliticorum (Barger & Esch, 1999)	*Notropis chiliticus* (Cope) *Clinostomus funduloides* Girard	Basin Creek, N.C. 6/18-19/12	NNXXX(3)	XXXXX
Plagioporus crookedensis n. sp.	*C. funduloides*	Crooked Creek, V.A. 6/20-21/12 8/17-18/13	NNXXX(3)	XXXXX
Plagioporus franksi n. sp	*Rhinichthys cataractae* (Valenciennes) *R. obtusus* Agassiz	Cosby Creek, T.N. 06/30/14	NNXXX(3)	XXXXX

Table 16 Sequences obtained from GenBank used for phylogenetic analysis.

Family	Species	Host	GenBank No.	Reference
Brachycladiidae	*Zalophotrema hepaticum* Stunkard & Alvey, 1929	*Zalophus californianus* (Lesson)	AY222255	Olson et al. (2003)
Acanthocolpidae	*Stephanostomum pristis* (Deslongchamps, 1824)	*Phycis phycis* (Linnaeus)	DQ248222	Bray et al. (2005)
Enenteridae	*Enenterum aurem* Linton, 1910	*Kyphosus vaigiensis* (Quoy & Gaimard)	AY222232	Olson et al. (2003)
Lepocreadiidae	*Preptetos caballeroi* Pritchard, 1960	*Naso vlamingii* (Valenciennes)	AY222236	Olson et al. (2003)
Opecoelidae	*Allopodocotyle epinepheli* (Yamaguti, 1942)	*Epinephelus cyanopodus* (Richardson)	KU320598	Bray et al. (2016)
Opecoelidae	*Allopodocotyle margolisi* Gibson, 1995	*Coryphaenoides mediterraneus* (Giglioli)	KU320596	Bray et al. (2016)
Opecoelidae	*Allopodocotyle* sp. A	*Scolopsis bilineata* (Bloch)	KU320599	Bray et al. (2016)
Opecoelidae	*Allopodocotyle* sp. B	*Epinephelus coioides* (Hamilton)	KU320607	Bray et al. (2016)
Opecoelidae	*Anomalotrema koiae* Gibson & Bray, 1984	*Sebastes viviparus* Krøyer	KU320595	Bray et al. (2016)
Opecoelidae	*Bathycreadium brayi* Pérez-del-Olmo, Dallarés, Carrassón & Kostadinova, 2014	*Trachyrincus scabrus* (Rafinesque)	JN085948	Constenla et al. (2011)
Opecoelidae	*Bentholebouria blatta* (Bray & Justine, 2009)	*Pristipomoides argyrogrammicus* (Valenciennes)	KU320608	Bray et al. (2016)
Opecoelidae	*B. blatta*	*Pristipomoides argyrogrammicus*	KU320606	Bray et al. (2016)
Opecoelidae	*Bentholebouria colubrosa* Andres, Pulis & Overstreet 2014	*Pristipomoides aquilonaris* (Goode & Bean)	KJ001207	Andres et al. (2014a)
Opecoelidae	*Biospeedotrema biospeedoi* Bray, Waeschenbach, Dyal, Littlewood & Morand (2014)	*Thermichthys hollisi* (Cohen, Rosenblatt & Moser)	KF733986	Bray et al. (2014)

Opecoelidae	*Biospeedotrema jolliveti* Bray, Waeschenbach, Dyal, Littlewood & Morand (2014)	*Ventichthys biospeedoi* Nielsen, Møller & Segonzac	KF733985	Bray et al. (2014)
Opecoelidae	*Buticulotrema thermichthysi* Bray, Waeschenbach, Dyal, Littlewood & Morand , 2014	*Thermichthys hollisi* (Cohen, Rosenblatt & Moser)	KF733984	Bray et al. (2014)
Opecoelidae	*Cainocreadium labracis* (Dujardin, 1845)	*Gibbula adansonii* (Payraudeau)	JQ694144	Born-Torrijos et al. (2012)
Opecoelidae	*Cainocreadium lintoni* (Siddiqi & Cable, 1960)	*Epinephelus morio* (Valenciennes)	KJ001208	Andres et al. (2014a)
Opecoelidae	*Dimerosaccus oncorhynchi* (Eguchi, 1931)	*Oncorhynchus masou* (Brevoort)	FR870252	Shedko et al. (2015)
Opecoelidae	*Gaevskajatrema halosauropsi* Bray & Campbell, 1996	*Halosauropsis macrochir* (Günther)	AY222207	Olson et al. (2003)
Opecoelidae	*Gaevskajtrema perezi* (Mathias, 1926)	Unidentified fish host	AF184255	Tkach et al. (2001)
Opecoelidae	*Hamacreadium mutabile* Linton, 1910	*Lutjanus griseus* (Linnaeus)	KJ001209	Andres et al. (2014a)
Opecoelidae	*Hamacreadium 'mutabile'*	*Lutjanus fulviflamma* (Forsskål)	KU320601	Bray et al. (2016)
Opecoelidae	*Hamacreadium* sp.	*Lethrinus miniatus* (Forster)	KU320603	Bray et al. (2016)
Opecoelidae	*Helicometra boseli* Nagaty, 1956	*Sargocentron spiniferum* (Forsskål)	KU320600	Bray et al. (2016)
Opecoelidae	*Helicometra epinepheli* Yamaguti, 1934	*Epinephelus fasciatus* (Forsskål)	KU320597	Bray et al. (2016)
Opecoelidae	*Helicometra manteri* Andres, Ray, Pulis, Curran & Overstreet, 2014	*Prionotus alatus* Goode & Bean	KJ701238	Andres et al. (2014b)
Opecoelidae	*H. manteri*	*Bellator egretta* (Goode & Bean)	KJ701239	Andres et al. (2014b)
Opecoelidae	*Maculifer* sp.	*Diodon hystrix* Linnaeus	AY222211	Olson et al. (2003)
Opecoelidae	*Macvicaria bartolii* Antar, Georgieva, Gargouri & Kostadinova, 2015	*Diplodus annularis* (Linnaeus)	KR149464	Antar et al. (2015)
Opecoelidae	*Macvicaria crassigula* (Linton, 1910)	*Calamus bajonado* (Black & Schneider)	KJ701237	Andres et al. (2014b)
Opecoelidae	*Macvicaria dubia* (Stossich, 1905)	*Oblada melanura* (Linnaeus)	KR149469	Antar et al. (2015)

Opecoelidae	*Macvicaria maamouriae* Antar, Georgieva, Gargouri & Kostadinova, 2015	*Sparus aurata* Linnaeus	KR149467	Antar et al. (2015)
Opecoelidae	*Macvicaria macassarensis* (Yamaguti, 1952)	*Lethrinus miniatus* (Forster)	AY222208	Olson et al. (2003)
Opecoelidae	*Macvicaria mormyri* (Stossish, 1885)	Unidentified fish host	AF184256	Tkach et al. (2001)
Opecoelidae	*Macvicaria obovata* (Molin, 1859)	*Cyclope neritea* (Linnaeus)	JQ694147	Born-Torrijos et al. (2012)
Opecoelidae	*Neolebouria lanceolata* Andres, Pulis & Overstreet, 2014	*Polymixia lowei* (Günther)	KJ001210	Andres et al. (2014a)
Opecoelidae	*Neoplagioporus ayu* (Takahashi, 1928)	*Plecoglossus altivelis altivelis* (Temminck & Schlegel)	XXXXXX	Fayton et al. (2016)
Opecoelidae	*Neoplagioporus elongatus* (Goto & Ozaki, 1930)	*Sarcocheilichthys variegatus microoculus* Mori	XXXXXX	Fayton et al. (2016)
Opecoelidae	*Neoplagioporus zacconis* (Yamaguti, 1934)	*Opsariichthys platypus* (Temminch & Schlegel)	XXXXXX	Fayton et al. (2016)
Opecoelidae	*Opecoeloides fimbriatus* (Linton, 1910)	*Micropogonias undulatus* (Linnaeus)	KJ001211	Andres et al. (2014a)
Opecoelidae	*Opecoeloides furcatus* (Bremser in Rudolphi, 1819)	*Mullus surmuletus* Linnaeus	AF151937	Tkach et al. (2000)
Opecoelidae	*Opistholebes amplicoelus* Nicoll, 1915	*Tetractenos hamiltoni* (Richardson)	AY222210	Olson et al. (2003)
Opecoelidae	*Pacificreadium serrani* (Nagaty & Abdel-Aal, 1962)	*Plectropomus leopardus* (Lacepède)	KU320602	Bray et al. (2016)
Opecoelidae	*Peracreadium idoneum* (Nicoll, 1909)	*Anarhichas lupus* Linnaeus	AY222209	Olson et al. (2003)
Opecoelidae	*Plagioporus aliffi* Fayton, Choudhury, McAllister & Robison, 2017	*Etheostoma blenniodies newmanni* Miller	KX905055	Fayton et al. (2017)
Opecoelidae	*P. boleosomi* (Pearse, 1924)	*Percina maculata* (Girard)	KX553953	Fayton et al. (2016)
Opecoelidae	*P. carolini* Fayton, McAllister & Connior, 201X	*Cottus carolinae* (Gill)	XXXXXX	XXXXXXXXXXXX
Opecoelidae	*P. chiliticorum* (Barger & Esch, 1999)	Notropis chiliticus (Cope)	KX553943	Fayton et al. (2016)

Opecoelidae	*P. fonti* Fayton, Choudhury, McAllister & Robsion, 2017	Percina nigrofasciata (Agassiz)	KX905054	Fayton et al. (2017)
Opecoelidae	*P. hageli* Fayton & Andres, 2016	Oncorhynchus mykiss (Walbaum)	KX553950	Fayton et al. (2016)
Opecoelidae	*P. ictaluri* Fayton, Robison, & McAllister, 201X	*Noturus lachneri* Taylor	XXXXXX	XXXXXXXXXXXXX
Opecoelidae	*P. kolipinskii* Tracey, Choudhury, Cheng & & Ghosh, 2009	*Gasterosteus aculeatus* Linnaeus	KX553952	Fayton et al. (2016)
Opecoelidae	*P. limus* Fayton, Choudhury, McAllister & Robison, 2017	*Etheostoma squamosum* Distler	KX905055	Fayton et al. (2017)
Opecoelidae	P. loboides (Curran, Overstreet, & Tkach, 2007)	*Fundulus nottii* (Agassiz)	EF523477	Curran et al. (2007)
Opecoelidae	*P. shawi* (McIntosh, 1939)	*Oncorhynchus tshawytscha* (Walbaum)	KX553951	Fayton et al. (2016)
Opecoelidae	*P. sinitsini* Mueller, 1934	*Notemigonus crysoleucas* (Mitchill)	KX553944	Fayton et al. (2016)
Opecoelidae	*Podocotyloides brevis* Andres & Overstreet, 2013	*Conger esculentus* Poey	KJ001212	Andres et al. (2014a)
Opecoelidae	*Pseudopecoeloides tenuis* Yamaguti, 1940	*Priacanthus hamrur* (Forsskål)	KU320605	Bray et al. (2016)
Opecoelidae	*Pseudopycnadena tendu* Bray & Justine, 2007	*Pseudobalistes fuscus* (Bloch & Schneider)	FJ788506	Bray et al. (2009)
Opecoelidae	*Propycnadenoides philippinensis* Fischthal & Kuntz, 1964	*Gymnocranius grandoculis* (Valenciennes)	KU320604	Bray et al. (2016)
Opecoelidae	*Urorchis acheiloghathi* Yamaguti, 1934	*Tanakia limbata* (Temminck &	KX553945	Fayton et al. (2016)
Opecoelidae	*Urorchis goro* Ozaki, 1927	*Rhinogobius* sp.	KX553946	Fayton et al. (2016)

Table 17 Pairwise comparisons of percent nucleotide similarity and the number of base pair differences (in parentheses) for the 28S, ITS-2, ITS-1 and 5.8S of species of *Plagioporus* provided in this study.

		Plagioporus franksi n. sp.	*Plagioporus fonti*	*Plagioporus limus*	*Plagioporus aliffi*	*Plagioporus boleosomi*	*Plagioporus chilliticorum*	*Plagioporus hageli*	*Plagioporus kolipinskii*	*Plagioporus sinitsini*	*Plagioporus shawi*	*Plagioporus loboides*	*Plagioporus carolini*	*Plagioporus ictaluri*
28S	*Plagioporus crookedensis* n. sp.	98.2 (24)	97.6 (32)	97.6 (31)	97.6 (32)	97.6 (32)	98.2 (25)	97.8 (29)	96.8 (42)	98.1 (25)	96.0 (52)	98.0 (24)	97.1 (38)	96.9 (41)
28S	*Plagioporus franksi* n. sp.	-	97.3 (36)	97.3 (36)	97.2 (38)	97.3 (36)	99.9 (1)	97.3 (36)	96.4 (48)	97.7 (30)	95.6 (57)	97.6 (29)	97.4 (34)	97.2 (37)
ITS2	*Plagioporus crookedensis* n. sp.	96.4 (9)	96.0 (10)	95.2 (12)	95.6 (11)	95.6 (11)	96.0 (10)	97.6 (6)	94.8 (13)	98.4 (4)	88.3 (28)	NA	96.8 (8)	96.4 (9)
ITS2	*Plagioporus franksi* n. sp.	-	95.2 (12)	92.4 (19)	94.4 (14)	94.4 (14)	99.6 (1)	96.4 (9)	93.5 (16)	96.4 (9)	87.5 (30)	NA	94.8 (13)	94.4 (14)
ITS1	*Plagioporus crookedensis* n. sp.	89.6 (68)	86.6 (89)	89.0 (71)	87.9 (80)	87.8 (81)	89.9 (66)	83.9 (106)	78.2 (144)	92.6 (47)	82.7 (85)	NA	88.2 (77)	88.1 (77)
ITS1	*Plagioporus franksi* n. sp.	-	85.2 (97)	86.5 (85)	85.1 (97)	85.4 (96)	99.7 (2)	82.9 (112)	76.4 (153)	87.2 (82)	81.4 (89)	NA	85.6 (95)	86.1 (91)
5.8S	*Plagioporus crookedensis* n. sp.	99.4 (1)	99.4 (1)	100 (0)	100 (0)	99.4 (1)	99.4 (1)	100 (0)	99.4 (1)	99.4 (1)	98.7 (2)	NA	98.1 (3)	98.1 (3)
5.8S	*Plagioporus franksi* n. sp.	-	98.7 (2)	99.4 (1)	99.4 (1)	98.7 (2)	100 (0)	99.4 (1)	100 (0)	98.7 (2)	99.4 (1)	NA	98.7 (2)	98.7 (2)

Figure 11. Plagioporus chiliticorum from the intestine of *Notropis chiliticus*. 1, Ventral view showing ventral vitelline fields; 2, Terminal genitalia, dorsal view; 3, Female complex, dorsal view Scale bars 100 μm for 1 and 50 μm for 2-3.

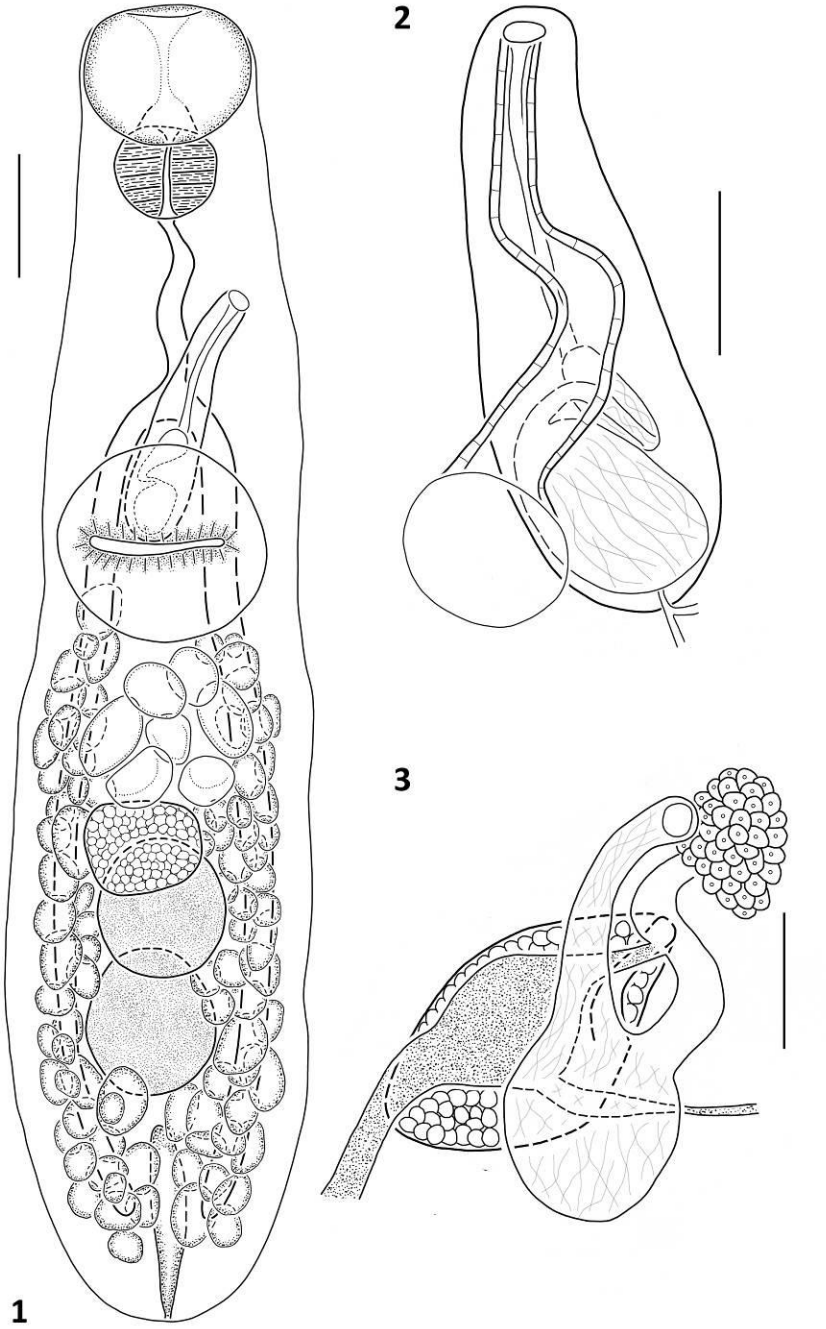


Figure 12. Plagioporus crookedensis n. sp. from the intestine of *Clinostomus funduloides*. 4, Ventral view showing ventral vitelline fields; 5, Dorsal view showing dorsal vitelline fields; 6, Terminal genitalia, lateral view; 7, Female complex, dorsal view. Scale bars: 100 μm for 4-5 and 50 μm for 6-7.

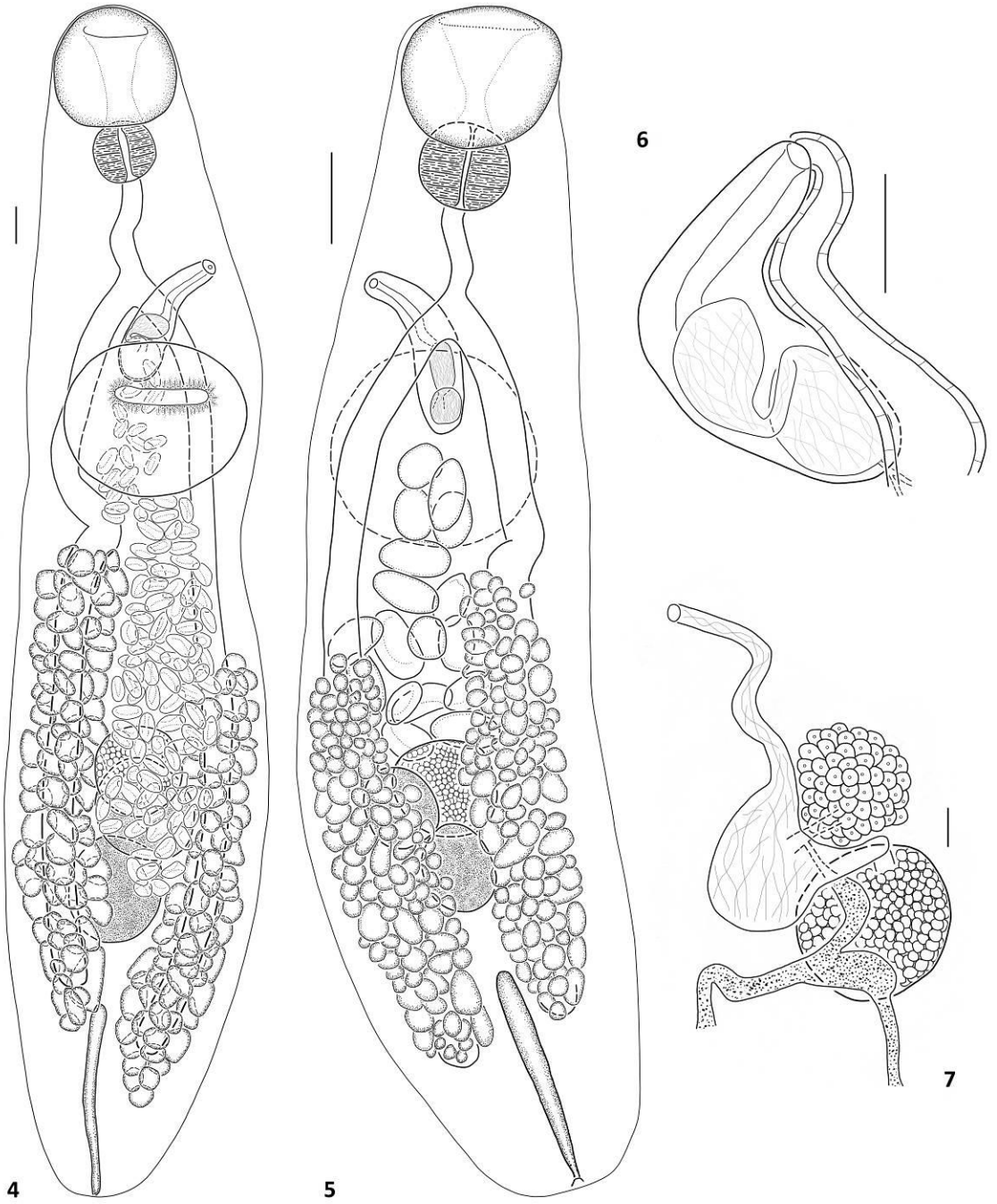


Figure 13. Plagioporus franksi n. sp. from intestine of *Rhinichthys cataractae*. 8, Ventral View; 9, Dorsal view showing dorsal vitelline fields; 10, Terminal genitalia, ventral view; 11, Female complex, dorsal view. Scale bars: 100 μm for 8-9 and 50 μm for 10-11.

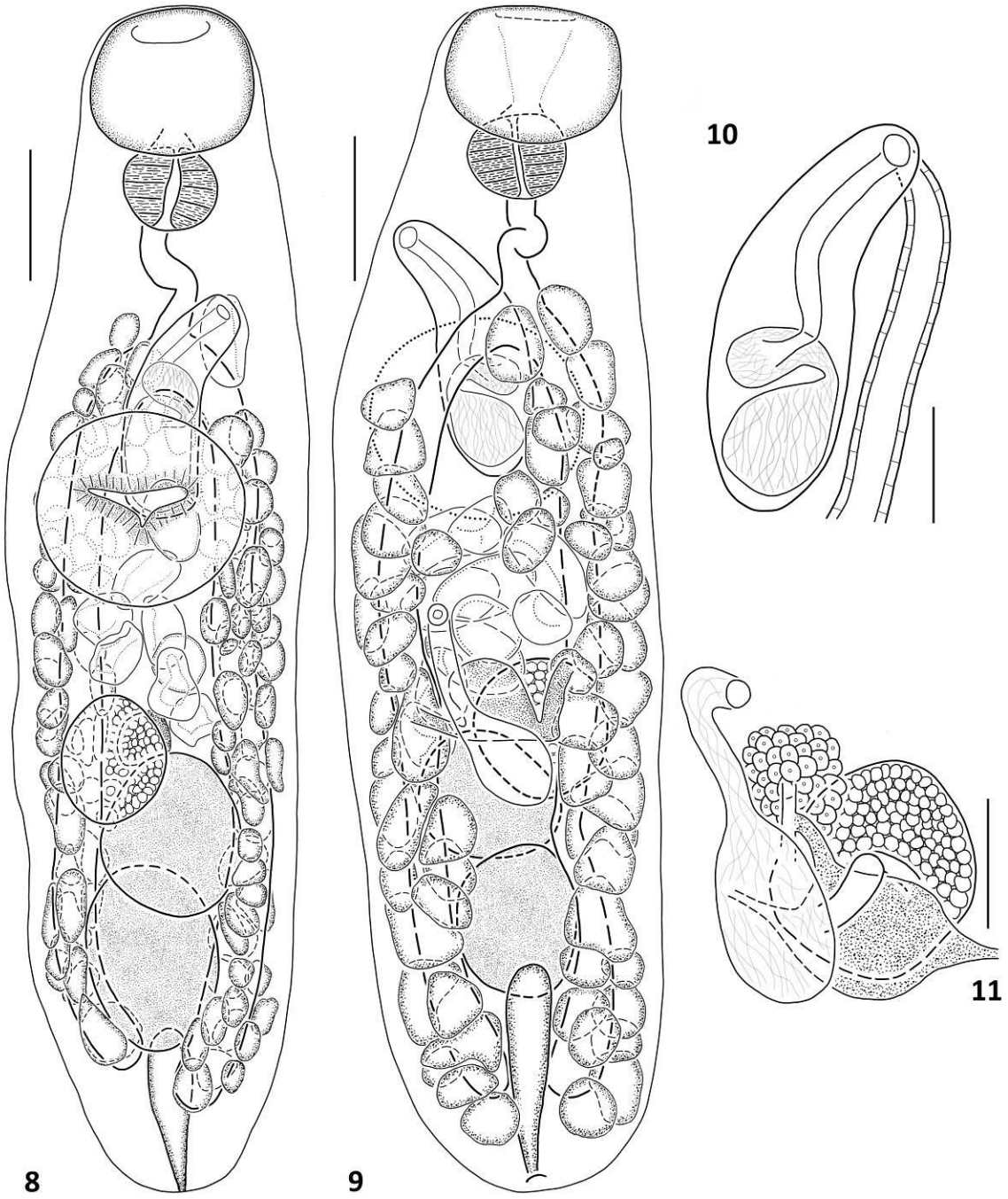
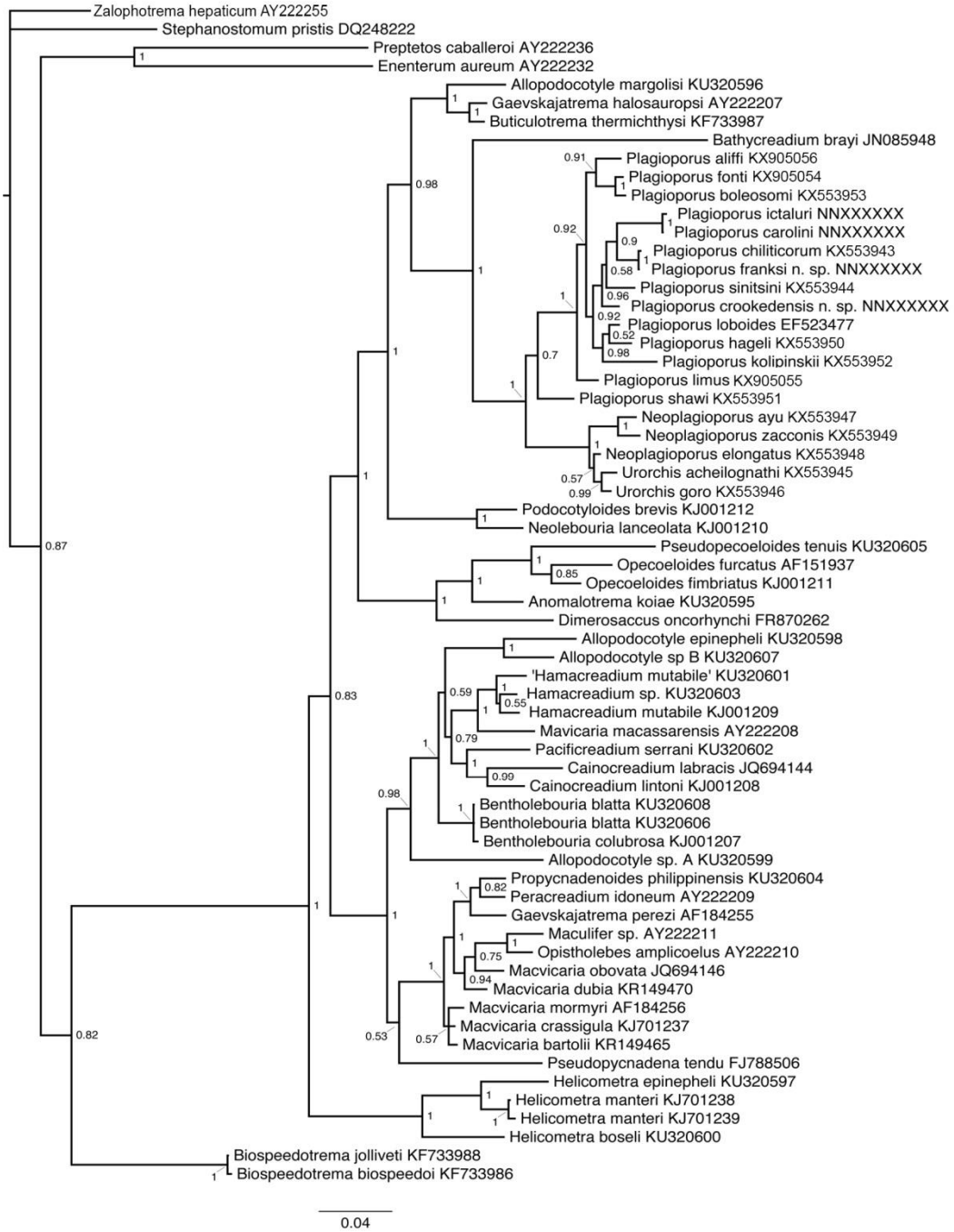


Figure 14. Phylogenetic relationships of opecoelids resulting from Bayesian inference analysis of partial 28S rDNA sequences (GTR + I + Γ) (5,000,000 generations and a sample frequency of 1,000).



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CHAPTER VI - Redescription of *Plagioporus serotinus* Stafford, 1904, with the description of two new species of *Plagioporus* Stafford, 1904 and *Plagioporus cf. hypentelii* from *Hypentelium nigricans* (Lesueur) from Tennessee and Arkansas and a larval form from Pennsylvania

Abstract

Plagioporus serotinus Stafford, 1904 is redescribed from type material and the redescription of Miller (1940) and its conspecificity with a form collected from the intestine of *Cyprinus carpio* Linnaeus from Montreal is assessed. In addition, three intestinal species of *Plagioporus* are described from *Hypentelium nigricans* as follows: *Plagioporus shirleyi* n. sp. from Crooked Creek, Arkansas, *Plagioporus hendrixi* n. sp. from Abrams Creek, Tennessee and *Plagioporus* cf *hypentelii* from Cosby Creek, Tennessee. These three species differ from Nearctic congeners in possession of wider than long oral and ventral suckers and having the vitellarium distributed from the intestinal bifurcation to nearly the end of the body, with an interruption at the level of the ventral sucker. *Plagioporus shirleyi* n. sp., *Plagioporus hendrixi* n. sp. and *Plagioporus cf hypentelii* are respectively distinguished from one another in the position of the ovary, possession of a lateral, sinistral bludge at the level of the genital pore, and having the testicular space occupy at least 50% of the hindbody. *Plagioporus* cf *hypentelii* was found to be most morphologically similar to *Plagioporus hypentelii*, the only species of *Plagioporus* previously known from *H. nigricans*, and while morphological differences were apparent, these could not be definitively attributed to interspecific variation as the type material (of *P. hypentelii*) was fixed in a contracted state, complicating morphological comparisons. We also describe a larval form of *Plagioporus* (*Plagioporus*

sp. A) from *Leptoxis carinata* (Brug) from Marsh Creek, Pennsylvania, the type locality of *H. nigricans*. Interestingly, ITS1, ITS2 and 28S rDNA sequences of *Plagioporus* cf *hypentelii* and *Plagioporus* sp. A revealed that the two forms are 100% identical and possibly conspecific. Bayesian inference analysis of two different allignments (28S rDNA only and 28S concatenated with ITS2) composed of sequence data of *Plagioporus* spp. collected in this study and that of other opecoelids obtained from GenBank revealed that the two new species, *Plaioporus* cf *hypentelii*, *Plagioporus* sp. A, and *P. sinitsini*, congeners known to infect catostomids with the exception of the larval form, composed a monophyletic clade within *Plagioporus* that was sister to another formed mostly by species parasitizing the intestine of cyprinids. Morphological examination of *Plagioporus* sp. A revealed that it may represent the larval form of *P. hypentelii*. We recommend that future work should sequence *P. hypentelii* from its type locality and host to assess its conspecificty with *Plagioporus* sp. A and *Plagioporus* cf *hypentelii*. Additionally, *P. serotinus* should be sequenced from all reported hosts in the St. Lawrence River drainage, including its type host, *Catostomus commersonii* (Lacepède) and *C. carpio*, and redescribed using specimens that are fixed in a relaxed state.

Introduction

Three species of intestinal *Plagioporus* Stafford, 1904 have been described from catostomids of the Nearctic. The type species of the genus, *Plagioporus serotinus*, Stafford, 1904 was described from the intestine of *Moxostoma macrolepidotum* (Lesueur) obtained from a fish market in Montreal, Quebec, Canada (Stafford, 1904) and was subsequently redescribed by Miller (1940) from *Moxostoma valenciennesi* Jordan (reported as '*Moxostoma aureolum* [red horse sucker]') and *Catostomus commersonii*

(Lacepède) from the Ottawa River near its confluence with the St. Lawrence River in Quebec, Canada. Haderlie (1953) reported *P. serotinus* from *Archoplites interruptus* (Girard) from Clear Lake, California, a specific identification questioned by Manter (1954) based on observed differences in egg size and shape of the cirrus sac. *P. serotinus* has also been reported from the gall bladder of *Pimephales notatus* (Rafinesque) (Aliff 1977) from Boone Creek, Kentucky and from *C. commersonii* (site of infection not specified) from the Kentucky River drainage (White 1974). Two additional intestinal species of *Plagioporus* have been described from catostomids, including *Plagioporus hypentelii* Hendrix, 1973 from *Hypentelium nigricans* (Lesueur) from Marsh Creek, Pennsylvania, and *Plagioporus macrouterinus* Haderlie, 1953 from *Catostomus macrocheilus* Girard from Deer Creek, California. *Plagioporus hypentelii* was later reported from *H. nigricans* from the Greenbrier River, West Virginia (Rubertone & Hall, 1975). An additional intestinal opecoelid, *Plagiocirrus testeus* Fritts, 1959 from *C. macrocheilus* from the Clearwater River, Idaho, may prove to be a species of *Plagioporus* sensu Fayton & Andres (2016).

During a survey of digenean parasites of freshwater fishes of Arkansas and Tennessee, three forms of *Plagioporus* from the intestine of *Hyptenlium nigricans* were recovered from the Great Smokey Mountains, Tennessee, and Crooked Creek, Arkansas, that differ from one another and known congeners. We also recovered larval *Plagioporus* from the snail *Leptoxis carinata* (Brug) from Marsh Creek, Pennsylvania. We describe the new adult and larval forms of *Plagioporus* using molecular and morphological methods, with former including the use ribosomal DNA sequence data to assess the phylogenetic relationships of the new forms with congeners and the latter including a

redescription of *P. serotinus* based on observation of specimens deposited in the Canadian Museum of Nature.

Material and Methods

On 23 July 2014, specimens of *Plagioporus* were collected from the intestine of *H. nigricans* from Crooked Creek, Arkansas (36° 13' 38.69"N 92° 42' 43.56"W) using a boat electroshocker. On 29-30 July 2014, 2 additional forms of *Plagioporus* from *H. nigricans* were sampled from the Great Smokey Mountains National Park via backpack electroshocker, including one from Cosby Creek, Tennessee (35° 46’ 39.94”N, 83° 12’ 46.75”W) and another from Abrams Creek, Tennessee (35° 36' 25.64"N, 83° 56' 4.73"W). On 20 August 2013, larval opecoelids were harvested from *Leptoxis carinata* (Brug) collected by hand from Marsh Creek, Pennsylvania (39° 51' 16.89"N, 77° 17' 15.14"W). From the same site, *Pimephales promelas* Rafinesque harboring adult intestinal opecoelids were collected via minnow trap and seine (Table 18). Specimens of opecoelids were removed from the intestine of fish hosts with the aid of a fine paintbrush and transferred to and observed in a shallow dish containing 0.6% saline. Two hundred *L. carinata* were isolated individually in stender dishes containing sufficient water collected from Marsh Creek (strained in 100 µm sieve) to immerse the snail hosts. Cercaria and sporocysts were transferred to and observed in a shallow dish containing habitat water and 0.6% saline, respectively. For adult worms, cercaria shed from snails, and sporocysts, most of the fluid was removed from the dish to the point where worms were restricted to the surface of the dish and attached to the glass by their suckers (for cercaria and adults), upon which near boiling (steaming hot) water was rapidly added to kill worms, minimizing contraction or curling post-fixation. Heat-killed worms were immediately

transferred to 10% neutral phosphate buffered formalin for morphological examination or 95% ethanol for molecular analysis. Worms were stained in acetocarmine or Mayer's or Ehrlich's haematoxylin, dehydrated in a graded ethanol series, cleared in methyl salicylate and mounted permanently in Canada balsam (Sigma-Aldrich) or Damar gum (Sigma-Aldrich). Helminth specimens collected during the present study were deposited in the collection of the Smithsonian National Museum of Natural History (NMNH), Washington, D.C. (Table 18). Specimens were examined using bright-field and Nomarski differential interference contrast (DIC) optics on an Olympus BX 51 microscope and illustrated using an attached drawing tube. The length of the internal seminal vesicle was determined by measuring the vesicle at its central axis, following its turns and loops. Measurements are given in micrometers (μm) unless otherwise specified and are expressed as the measurements of the holotype followed by the minimum and maximum values of paratypes in parentheses. *Plagioporus serotinus* is redescribed from 3 specimens prepared by Stafford, 1904, with measurements consisting of the minimum and maximum values of these specimens. The length and width of vitelline follicles are expressed as the minimum and maximum values of 10 random follicles distributed throughout the body. Characters expressed as a measurement followed by body length (BL) refer to the distance from the anterior end. For the redescription of *P. serotinus* from type material, minimum and maximum values of specimens are presented in Table 20 along with Stafford's (1904) original measurements and those from the redescription of Miller (1940). Additional measurements were made from the line drawings of Miller's (1940) redescription and specimens of opecoelids from the intestine of *Cyprinus carpio* Linnaeus identified by Webster in 1976 as *P. serotinus* (Table 20). Material was

examined from the Canadian Museum of Nature as follows: XXXXX. Material was examined from the NMNH as follows: 72459, paratype of *P. hypentelii*.

Genomic DNA was isolated from each species of *Plagioporus* (number of replicates [from separate individual worms] per species displayed in Table 18) using Qiagen DNAeasy Tissue Kit (Qiagen, Inc., Valencia, California, USA) following the instructions provided by the manufacturer. DNA fragments c. 2,550 base pairs (bp) long, comprising the 3′ end of the 18S nuclear rDNA gene, internal transcribed spacer regions, ITS1 and ITS2 (including 5.8S) and a partial sequence of the 28S rDNA gene (including variable domains D1–D3), were amplified from the extracted DNA by polymerase chain reaction (PCR) on a PTC-200 Peltier Thermal Cycler using forward primer ITSF (5′ CGC CCG TCG CTA CTA CCG ATT G-3′) and reverse primer 1500R (5′ GCT ATC CTG AGG GAA ACT TCG-3′). These PCR primers and multiple internal primers were used in sequencing reactions. The internal forward primers were digl2 (5′ AAG CAT ATC ACT AAG CGG-3′), 300F (5′ CAA GTA CCG TGA GGG AAA GTT G-3′) and 900F (5′ CCG TCT TGA AAC ACG GAC CAA G-3′) and the internal reverse primers were 300R (5′ CAA CTT TCC CTC ACG GTA CTT G-3′), digl2R (5′ CCG CTT AGT GAT ATG CTT-3′) and ECD2 (5′ CTT GGT CCG TGT TTC AAG ACG GG-3′) (for primers see Littlewood et al., 2000; Tkach et al., 1999, 2000, 2001, 2003; Tkach & Snyder, 2007). The resulting PCR products were excised from PCR gels using QIAquick Gel Extraction Kit (Qiagen, Inc., Valencia, California, USA) following the manufacturer's instructions, cycle-sequenced using ABI BigDye™ chemistry (Applied Biosystems, Inc., Carlsbad, California, USA), ethanol-precipitated, and run on an ABI 3130 Genetic Analyzer™. The sequences of the three new species herein described were assembled using Sequencher™

(GeneCodes Corp., Ann Arbor, Michigan, USA, Version 4.10.1) and deposited in GenBank (Table 1). The sequences were aligned using MAFFT version 6.611b (Katoh et al., 2005) with 1,000 cycles of iterative refinement and the genafpair algorithm. The boundaries between the 5.8S, ITS2 and 28S genes were located using the ITS2 Ribosomal Database (Keller et al., 2009). Pairwise sequence comparisons of the ITS1, 5.8S, ITS2 and 28S nuclear rDNA genes of the three new species of *Plagioporus* from this study and available sequences of *Plagioporus* from GenBank were calculated with MEGA v6 with gaps treated as missing data (Table 24). For phylogenetic analysis, sequences of opecoelids were obtained from GenBank (Table 19). The resulting alignments utilized the brachycladiid *Zalophotrema hepaticum* Stunkard & Alvey, 1929 as the outgroup based on its phylogenetic position relative to the Opecoelidae (Olson et al., 2003). Phylogenetic analysis of the concatenated complete ITS2 and partial 28S rDNA gene and 28S rDNA gene alone was performed using Bayesian Inference (BI) with MrBayes 3.2.6 software (Huelsenbeck & Ronquist, 2001) run on the CIPRES portal (Miller et al., 2010) (Figure 20). The best nucleotide substitution model for both of these genes (concatenated and each gene individually) was estimated with jModeltest-2 (Darriba et al., 2012) as general time reversible with estimates of invariant sites and gamma-distributed among site-rate variation (GTR + I + Γ). The following model parameters were used in MrBayes: nst = 6, rates = invgamma, ngen = 5,000,000 and samplefreq = 1,000. Burn-in value was 4,000 estimated by plotting the log-probabilities against generation and visualizing plateau in parameter values (sump burnin = 4,000), and nodal support was estimated by posterior probabilities (sumt) (Huelsenbeck et al., 2001) with all other settings left as default.

Redescription of *Plagioporus serotinus* Stafford, 1904 Miller, 1940

Plagioporus serotinus Stafford, 1904 Miller, 1940

Description (Fig. 15.1-2, Table 20)

[Measurements based on 3 non-gravid wholemounts.] Body lanceolate, with bluntly rounded ends, widest at approximately 1/3 of body length (BL), 1,264-1,624 long, 345-435 wide. Oral sucker subequal, subterminal, 128-157 long, 115-148 wide. Ventral sucker wider than long, 177-256 long, 188-271 wide; width representing 54-77 of% body width (BW). Forebody 371-517, representing 29-35% of BL. Ratio of oral sucker to ventral sucker width 1:1.6–1.9. Prepharynx 14, conspicuous or absent in 2 specimens. Pharynx slightly separated from to contiguous with oral sucker, subequal, 66-98 long, 63-89 wide. Oesophagus 87-107 long (n=2). Intestinal bifurcation anterior to ventral sucker at 273-325 BL, representing 20-22% of BL (n=2); post-caecal space143 long, representing 9% of BL (n=1).

Testes 2, tandem to slightly oblique, contiguous to slightly overlapping; anterior testis subequal, 139-182 long, 163-216 wide, with anterior margin at 757-910 BL, representing 56–60% of BL (n=2); posterior testis subequal, 139-212 long, 135-214 wide, dorsal to anterior testis, with anterior margin at 899-1,063 BL, representing 65-71% of BL (n=2). Post-testicular space 192-382, representing 19–22% of BL. Cirrus-sac clavate, 321 long, 68 wide, overlapping anterior 1/4 of ventral sucker, representing 20% of BL (n=1). Internal seminal vesicle 101 long, 48 wide, occupying posterior 31% length of sac. Ejaculatory duct present, not clearly differentiated from pars prostatica. Genital pore

ventrolateral, sinistral, 282-385 from anterior end of body, representing 21-22% of BL (n=2).

Ovary ovoid, subequal, 83-110 long, 89-92 wide, dextrally oblique to anterior testis, slightly overlapping to contiguous with anterior testis, overlapping dextral caecum ventrally, with anterior margin at 671-892 BL, representing 53-55% of BL (n=2). Mehlis' gland immediately anterior to anterior testis. Uterus conspicuous. Vitellarium follicular, mostly ventral to caeca with two dorsal fields at level of ventral sucker and in posttesticular space, in 2 lateral fields anterior to posterior margin of posterior testis and confluent or not in post-testicular space, anterior extent 305-316 from anterior end, representing 19–5% of BL; posterior extent at 1,195-1,476 BL or 91–95% of BL (n=2); length of follicles 12-44, width 8-58 (n=1). Vitelline reservoir conspicuous.

Excretory vesicle I-shaped, sac-like, dorsal to vitellarium and testis, extending anteriorly to posterior 1/4 of posterior testis, 443 long, representing 27% of BL, 44 wide (n=1); pore terminal.

Remarks

Stafford's (1904) description of *P. serotinus* was very brief and based on a single specimen without eggs. Stafford (1904) submitted a single slide of *P. serotinus* from *M. macrolepidotum* to the Canada Museum of Nature containing the type specimen and two additional specimens, all of which were non-gravid, fixed under coverslip pressure, and intensely stained with carmine, obscuring some of the features. My redescription is based on measurements of these 3 specimens (Table 20, Fig.15.1-2). My measurements of the type specimen and those of Stafford (1904) are mostly consistent, with the dimensions of the body and oral sucker being nearly identical. Stafford (1904) reported the dimensions

of the ventral sucker as a diameter (231 µm), implying a width and length that are equal to one another; however, my measurement of the type specimen yields a ventral sucker that is 223 µm long by 251 µm wide. This discrepancy led to a difference in the ratio of oral to ventral sucker width between our measurements and those of Stafford (1904) (1:1.9 as opposed to 1:1.5, respectively). There was also a slight difference in the percent body length of the forebody (32% of BL from our measurements as opposed to 40% of BL reported by Stafford [1904]). Moreover, Stafford (1904) reports the oesophagus length as twice the length of the pharynx, whereas my measurements of the type specimen yield an oesophagus that is 1.4 times the length of the pharynx. Lastly, Stafford (1904) reports the vitellarium distribution as lateral. I observed a median vitelline field that unties the lateral fields in the posttesticular space. Interestingly, this median field was not observed in all of Stafford's (1904) specimens. This variation could be caused by coverslip pressure displacing the median field laterally, an artifact of the intense staining of the specimens that effectively obscured many features, or intraspecific variation. The former 2 possibilities seem more likely given Miller's (1940) redescription of *P. serotinus* that reports that vitellarium as becoming confluent in the posttesticular space.

Miller's (1940) redescription of *P. serotinus* from the intestine of *C. commersonii* and *M. valenciennesi* is consistent with my measurements of Stafford's (1904) slides and those of the original description. There is a slight difference in the ratios of body length to body width and oral sucker to ventral sucker width, but Miller (1940) notably reports these characters as approximate. The body length of specimens of *P. serotinus* from *C. commersonii* can be smaller than that of *P. serotinus* from *M. macrolepidotum* and conversely, specimens from *M. valenciennesi* can be longer than of specimens prepared

by Stafford (1904) (Miller, 1940). Given that the redescription of Miller (1940) is brief, I report additional measurements of the 2 specimens that he illustrated in Table20. Unfortunately Miller (1940) did not specify the host(s) of the 2 specimens illustrated. Based on the reported body lengths for *P. serotinus* from the 2 catostomid species examined, I infer that Figure 3 of Miller (1940) is a specimen from *M. valenciennesi*. I suspect that Figure 2 of Miller (1940) is a specimen from *C. commersonii*, though this will have to be confirmed through examination of deposited slides, if available. My measurements of the two specimens illustrated by Miller (1940) are consistent with those of my redescription and Stafford's (1904) specimens. The only significant differences between my measurements of Miller's (1940) specimens and my measurements of Stafford's specimens are the length of the oesophagus (4% (n=1) compared with 7% (n=2) of BL, respectively) and length of the excretory vesicle (16% (n=1) versus 27% (n=1) of BL, respectively). We note that while there was a difference in the percent BL of the excretory vesicle, the relative extent of the vesicle to the posterior testis was identical in the 2 specimens available for comparison, and the specimen illustrated by Miller (1940) was shorter (1,229 µm versus 1,624 µm).

In Table 20, measurements of 4 specimens collected from the intestine of *Cyprinus carpio* Linnaeus from Montreal, Canada identified by Webster in 1976 as '*Plagioporus serotinus*' are included. The original labels to these slides appear to have been written by Stafford. While is possible that this invasive host species was misidentified (perhaps being confused with *Carpiodes cyprinus* [Lesueur]), one intestinal species of *Plagioporus* is a generalist of cyprinid hosts (Hunter & Bangham, 1932) and one species, *Plagioporus sinitsini* Mueller, 1934, is known to parasitize both catostomids

and cyprinids (Dobrovolny, 1939a). The measurements of the 4 specimens from *C. carpio* largely overlap the range of measurements reported by Miller (1940), Stafford (1904), my redescription of *P. serotinus* and my measurements of the line drawings of Miller (1940), but differ significantly in several features (Table 20). The ratio of the body length to body width, anterior testis width (expressed as a percent of BL), postcaecal space and ratio of the cirrus sac length to width is smaller in the worms from *C. carpio*. Moreover, the ovary width expressed as a percent of BW is wider and the anterior extent of the vitellarium more anterior in the specimens of '*P. serotinus*' from *C. carpio*. I also note that the vitellarium was not confluent in the postesticular space in the specimens from *C. carpio*. Given these differences, it is possible that the specimens from *C. carpio* are not conspecific with *P. serotinus*. Conversely, these differences could be host specific, represent intraspecific variation, be caused by a difference in fixation, or a combination thereof. I recommend that future studies utilize sequence data to compare both forms and include morphological data from a greater number of specimens than was available for this study.

I agree with Manter (1954) that Haderlie's (1953) report of *P. serotinus* from *Archoplites interruptus* (Girard) from Clear Lake, California is a misidentification. Manter (1954) notes differences in egg size and cirrus shape between Haderlie's (1953) specimens and those of *P. serotinus*. Parasitism of centrarchids by *Plagioporus* is rare in the Nearctic; only a single species has a centrarchid host and it has only been reported from Michigan and Canada (Chapmann et al., 2015; Dobrovolny, 1939b; Hazen & Esch, 1977). It seems more likely that a form of *Plagioporus* from *Archoplites interruptus*, the only extant centrarchid with a native distribution West of the Rocky Mountains, would

represent a new species as opposed to *P. serotinus*, which has only been found as far West as Kentucky (White, 1974). I also suspect that Aliff's (1977) report of *P. serotinus* from the gall bladder of *Pimephales notatus* (Rafinesque) (Aliff 1977) from Boone Creek, Kentucky River drainage, Kentucky, is a misidentification. This is the only report of *P. serotinus* from the gall bladder, as opposed to the intestine, of its fish host (Stafford, 1904; Miller, 1940; White, 1974). I have collected a form of *Plagioporus* from the gall bladder of *P. notatus* from North Elkhorn Creek, Kentucky River drainage, Kentucky and intend to describe it as new in a forthcoming publication. This species is much more similar to *P. sinitsini*, a known gall bladder plagioporid, than it is to *P. serotinus*, particularly in the ratio of body length to width, extent of the excretory vesicle, posttesticular space and distribution of the vitellarium (TJF [unpublished observations]).

On 31 May through 1 June 2016, I examined 32 *C. commersonii* collected from the St. Lawrence River (Îlet Vert at 45° 42' 23.0"N, 73° 27' 14.3"W) that had been collected the previous week. While these hosts were infected with *Lissorchis* sp., no *P. serotinus* was found. I examined an additional 75 *P. serotinus* that were purchased from Lachine Bait Shop in Lachine, Quebec, Canada. The source of these fish was purportedly the St. Lawrence River. While these fish were infected with *Lissorchis* sp. and *P. sinitsini*, they were negative for *P. serotinus*. On 2 June 2016, a single adult *M. macrolepidotum* from the St. Lawrence River (Sorel-Tracey at 46º 02' 88.1"N, 73º08' 12.1"W.) approximately 61 cm was dissected. It too was negative for *P. serotinus*.

Plagioporus serotinus can be distinguished from *Plagioporus chiliticorum* (Barger & Esch, 1999) Cribb, 2005, *Plagioporus crookedensis* Fayton, 201X, *Plagioporus franksi* Fayton, 201X, *P. sinitsini*, *Plagioporus serratus* Miller, 1940,

Plagioporus loboides (Curran, Overstreet & Tkach, 2007) Fayton, 2016, *Plagioporus carolini* Fayton, McAllister, Robison, & Connior and *Plagioporus ictaluri* Fayton, Robison, & McAllister, 2016 in possession of a median vitelline field in the posttesticular space; from *Plagioporus fonti* Fayton, Choudhury, McAllister, & Robison, 2016, *Plagioporus limus* Fayton, Choudhury, McAllister, & Robison, 2016, *Plagioporus aliffi* Fayton, Choudhury, McAllister, & Robison, 2016, *Plagioporus boleosomi* (Pearse, 1924) Peters, 1957 and *Plagioporus lepomis* in having the vitellarium distributed as far anteriorly as the level of the intestinal bifurcation; from *Plagioporus macrouterinus* in lacking a uterus that extends posteriorly to the posterior testis; from *P. hypentelii* in having the testicular space occupy 38-40% of the length of the hindbody (as opposed to over 50% the length of the hindbody); from *Plagioporus cooperi* (Hunter & Bangham, 1932) Price, 1934 in having the intestinal bifurcation between the suckers as opposed to at the level of to posterior to the anterior margin of the ventral sucker; from *Plagioporus hageli* Fayton & Andres, 2016 and *P. shawi* (McIntosh, 1939) Margolis, 1970 in lacking a consistent interruption in the distribution of the vitellarium; from *P. siliculus* Sinitsin, 1931 in having an excretory vesicle that extends to the level of the posterior testis as opposed to that of anterior testis; and from *P. kolipinskii* Tracey, Choudhury, Cheng, & Ghosh, 2009 in possession of a ventral sucker that occupies 54-77% of the BW as opposed to one almost occupying the entire body width. *P. serotinus* is most similar to *P. shawi*, *P. siliculus*, *P. kolipinskii* and *P. hageli* in the body length-to-width ratio and in having the vitellarium confluent in the post-testicular space and extending anteriorly to approximately the level of the intestinal bifurcation. *P. serotinus* can be further distinguished from these congeners as follows: from *P. hageli* in the presence of dorsal

vitelline fields; from *P. kolipinskii* in having the cirrus sac represent 20-21% of BL compared with 7-13% of BL; from *P. shawi* in lacking an excretory vesicle that reaches the level of the ovary; and from *P. siliculus* in having a cirrus sac that overlaps the ventral sucker.

Description of *Plagioporus shirleyi* n. sp.

Plagioporus shirleyi n. sp.

Type- and only known-host: *Hypentelium nigricans* (Lesueur), Northern Hog Sucker (Cypriniformes: Catostomidae)

Type-locality: Crooked Creek, Marion County, Arkansas, U.S.A. (36° 13' 38.69"N 92° 42' 43.56"W)

Site in host: Intestine.

Prevalence: 1 of 2 hosts (50%).

Intensity: 40.

Type-material: Holotype (USNM XXXXXXX), Paratype (USNM XXXXXXX-X).

Representative DNA sequences: Partial (D1–D3) 28S: GenBank accession no. NNXXXXXX, from 3 identical sequences (from separate individual worms).

Etymology: This species is named after Kenneth E. Shirley**,** retired veteran district fisheries biologist of the Arkansas Game and Fish Commission, for his career's work and unselfish efforts in helping us sample catostomids.

Description (Fig. 16.3-6)

[Measurements based on 10 gravid wholemounts from *Hypentelium nigricans*] Body white to yellow in life, elongate cylindrical to elongate lanceolate, tapering anteriorly, tapering gradually in posterior 3/5, widest at approximately 1/3 of body length (BL), 1,343 (1,085-1,480) long, 283 (221-320) wide. Oral sucker subterminal, wider than long, 102 (88-120) long, 122 (106-128) wide. Ventral sucker wider than long, 164 (154-178) long, 215 (186-229) wide; width representing 76 (69-84)% of body width. Forebody 356 (271-367), representing 27 (25-27)% of BL. Ratio of oral sucker to ventral sucker width 1:1.8 (1:1.5-1.9). Prepharynx 12 (3-11) long. Pharynx wider than long, slightly overlapping oral sucker to overlapping it by 1/3 length, 55 (43-60) long, 66 (50-68) wide. Oesophagus 79 (60-97) long, representing 6 (4-7)% of BL, with or without slight turn. Intestinal bifurcation 1/2 to 3/4 distance between suckers at 225 (193-241), representing 17 (14-19)% of BL; postcaecal space 98 (81-114) long, representing 7 (6-8)% of BL.

Testes 2, tandem; anterior testis subequal, 165 (145-181) long, 169 (141-175) wide, overlapping caecae ventrally, with anterior margin at 763 (616-838) BL, representing 57 (54-58)% of BL; posterior testis longer than wide, 208 (168-253) long, 165 (138-195) wide, dorsal to anterior testis, overlapping anterior testis slightly to separated from it by 2, with anterior margin at 910 (748-1,041) BL, representing 68 (65-71)% of BL. Postesticular space 232 (127-330), representing 17 (12-22)% of BL. Cirrus sac clavate, 241 (190-262) long, representing 18 (14-19) % of BL, 73 (56-81) wide, overlapping anterior 1/5–1/2 of ventral sucker. Cirrus eversible. Vasa efferentia uniting vas deferens at proximal end of cirrus sac. Internal seminal vesicle sac-like, 123 (110-145) long, representing 51 (52-73)% length of cirrus sac, 70 (45-67) wide, communicating with thick-walled *pars prostatica*; pars prostatica s-shaped to winding,

communicating with indistinct tubular region likely representing ejaculatory duct in anterior ½ of cirrus sac. Genital pore ventrolateral, sinistral, 221 (166-212) from anterior margin of body, representing 16 (14-18)% of BL.

Ovary ovoid, subequal, 91 (72-105) long, 111 (68-109) wide, dextrally oblique to anterior testis, overlapping anterior testis slightly to in posterior 1/3 of length, ventrally overlapping dextral caecum, with anterior margin at 691 (562-759) BL, representing 51 (50-53)% of BL. Postovarian space 569 (426-644), representing 42 (39-44) % of BL. Oviduct extending anterodorsally from anterior portion of ovary, turning posteriorly to laterally to join with canalicular seminal receptacle; seminal receptacle median, sac-like, dorsal to anterior testis, extending posteriorly to anterior 1/4 of anterior testis. Laurer's canal not clearly distinguished from seminal receptacle, bulbous, opening sinistrally on dorsal surface slightly posterior to ventral sucker. Mehlis' gland median, overlapping ovary by 1/2 length or anterior to it. Ootype extending anteriorly to laterally from seminal receptacle, conspicuous at level of Mehlis' gland. Uterus preovarian to extending posteriorly to anterior 1/3 anterior testis length, containing 43 (30-45) eggs. Metraterm arising slightly posterior to ventral sucker, weakly muscular, joining distal end of ejaculatory duct at genital pore. Eggs 62 (58-64) long, 32 (29-39) wide. Vitellarium follicular, ventral and dorsal to caeca, with interruptions in distribution at the level of ventral sucker and testicular space, with or without interruption at level of uterus, confluent field in postesticular space; ventral field dense at level of caecal bifurcation and extending to anterior half of ventral sucker, distributed laterally in hindbody anterior to posterior margin of posterior testis, forms confluent field in posttesticular space or not; dorsal field dense at level of caecal bifurcation and extending to anterior half of ventral

sucker, scattered laterally in hindbody anterior to testis, confluent field extending from testis to nearly the end of the body, median to spanning the body width. Vitellarium with anterior extent at to slightly anterior to intestinal bifurcation, 203 (164-207) from anterior end, representing 15 (13-16)% of BL, posterior extent at 1,282 (1,022-1,411), representing 95 (94-97)% of BL. Follicles of vitellarium number 155 (136-179), 21-59 (19-59) long, width 18-41 (16-62) wide. Vitelline reservoir median, dorsal to anterior testis, ventral to seminal receptacle. Common vitelline duct joining ootype at level of Mehlis' gland.

Excretory vesicle I-shaped, sac-like, posterior to posterior testis, 89 (65-103) long, representing 7 (5-7)% of BL, 27 (12-28) wide; pore terminal

Remarks

Plagioporus shirleyi n. sp. can be distinguished from *P. chiliticorum*, *P. crookedensis*, *P. franksi*, *P. sinitsini*, *P. serratus*, *P. loboides*, *P. carolini* and *P. ictaluri* in possession of a median vitelline field in the posttesticular space; from *P. fonti*, *P. limus*, *P. aliffi*, *P. boleosomi* and *P. lepomis* in having the vitellarium distributed as far anteriorly as the level of the intestinal bifurcation; from *P. serotinus*, *P. siliculus*, *P. hypentelii*, *P. cooperi* and *P. macrouterinus* in possession of a consistent interruption of the vitellarium at the level of the ventral sucker, and from *P. hageli*, *P. kolipinskii* and *P. shawi* in having an excretory bladder that never reaches the posterior testis. *Plagioporus shirleyi* n. sp. is most similar to *P. serotinus*, *P. kolipinskii* and *P. hageli* in body length and possession of vitellarium confluent in the post-testicular space and extending anteriorly to approximately the level of the intestinal bifurcation, but can be distinguished from these species as follows: from *P. serotinus* in having an excretory vesicle that never

reaches the posterior testis and smaller eggs (58-64 µm versus 70-90 µm long); from *P. kolipinskii* in possession of a longer cirrus sac (14-19% versus 7-13% of BL) and lack of a ventral sucker that occupies almost all of the body width; and from *P. hageli* in having the vitellarium ventral and dorsal to the caecae and a shorter forebody (25-27% compared with 31-38% of BL).

Plagioporus shirleyi n. sp. was not found in *Moxostoma erythrurum* (Rafinesque), centrarchids, ictalurids, fundulids, and cyprinids from the type locality (Table 21). In addition to Crooked Creek, we also found the new species in *H. nigricans* from Poke Bayou (North of Batesville off US 69, Independence Co., Arkansas, 35° 47' 30.7278" N, 91° 38' 41.6322"W), which like the type locality is also a tributary of the White River. Cyprinids, cottids, and percids from Poke Bayou were negative for *Plagioporus shirleyi* n. sp. (Table 21).

Description of *Plagioporus hendrixi* n. sp.

Plagioporus hendrixi n. sp.

Type- and only known-host: *Hypentelium nigricans* (Lesueur), Northern Hog Sucker (Cypriniformes: Catostomidae)

Type-locality: Abrams Creek, Blount Co., Tennessee, U.S.A. (35° 36' 25.64"N, 83° 56' 4.73"W)

Site in host: Intestine.

Prevalence: 3 of 3 hosts (100%).

Intensity: 9-21 per host (average 12).

Type-material: Holotype (USNM XXXXXXX), Paratype (USNM XXXXXXX-X).

Representative DNA sequences: Complete ITS1 and ITS2 regions, 5.8S gene, partial (D1–D3) 28S: GenBank accession no. NNXXXXXX, from 3 identical sequences (from separate individual worms).

Etymology: This species is named after Sherman S. Hendrix**,** retired professor of Gettysburg College, in recognition of his previous work on *Plagioporus*, helminth surveys of fishes of the Great Smokey Mountains National Park, and assistance with TJF's sampling efforts in Pennsylvania.

Description (Figs. 17.7-10)

[Measurements based on 10 gravid wholemounts from *Hypentelium nigricans*] Body white to yellow in life, elongate lanceolate, tapering anteriorly, tapering gradually in posterior 1/2 to 3/5, widest at approximately 1/3 to 2/5 of body length (BL), 1,250 (964-1252) long, 294 (186-296) wide. Oral sucker subterminal, wider than long, 117 (82-136) long, 139 (109-149) wide. Ventral sucker wider than long, 175 (145-194) long, 236 (188-237) wide; width representing 80 (73-86)% of body width. Forebody 353 (289-384), representing 28 (27-31)% of BL, asymmetrical, with sinistral, lateral bulge in tegument approximately at 1/5 length of body . Ratio of oral sucker to ventral sucker width 1:1.7 (1:1.5-1.8). Prepharynx 8 (6-16) long. Pharynx wider than long, slightly separated from oral sucker to overlapping it by 1/4 length, 57 (40-58) long, 66 (52-68) wide. Oesophagus 92 (55-93) long, representing 7 (6-8)% of BL, with or without slight turn. Intestinal

bifurcation 1/2 to 2/3 distance between suckers at 239 (180-246), representing 19 (19-21)% of BL; postcaecal space 97 (52-112) long, representing 8 (5-9)% of BL.

Testes 2, tandem; anterior testis subequal, 139 (123-148) long, 146 (120-151) wide, overlapping caecae ventrally, with anterior margin at 855 (625-769) BL, representing 68 (60-65)% of BL; posterior testis subequal, 131 (128-168) long, 138 (117-152) wide, dorsal to anterior testis, overlapping anterior testis slightly, with anterior margin at 989 (752-910) BL, representing 79 (71-78)% of BL. Postesticular space 129 (66-187), representing 10 (7-16)% of BL. Cirrus sac clavate, 323 (213-306) long, representing 26 (19-25) % of BL, 86 (52-90) wide, overlapping ventral sucker by 1/2 to all of its length. Cirrus eversible. Vasa efferentia uniting vas deferens at proximal end of cirrus sac. Internal seminal vesicle sac-like, 261 (163-274) long, representing 81 (74-92)% length of cirrus sac, 68 (32-89) wide, communicating with indistinct tubular region likely representing *pars prostatica* and ejaculatory duct in anterior 1/4 of cirrus sac. Indistinct tubular region with a turn. Genital pore ventrolateral, sinistral, 218 (191-240) from anterior margin of body, representing 17 (17-20)% of BL.

Ovary ovoid to tear drop-shaped, subequal, 120 (99-117) long, 84 (78-96) wide, dextrally oblique to anterior testis, overlapping anterior testis in posterior 1/4-1/2 of length, ventrally overlapping dextral caecum, with anterior margin at 761 (573-700) BL, representing 61 (54-60)% of BL. Postovarian space 385 (295-483), representing 31 (31-39) % of BL. Oviduct extending anterodorsally from anterior portion of ovary, turning posteriorly to laterally to join with canalicular seminal receptacle; seminal receptacle median, sac-like, dorsal to anterior testis, extending posteriorly to posterior 1/3 of ovary. Laurer's canal not clearly distinguished from seminal receptacle, bulbous, opening

sinistrally on dorsal surface slightly posterior to ventral sucker. Mehlis' gland median, slightly overlapping to anterior to ovary. Ootype extending anteriorly from seminal receptacle, conspicuous at level of Mehlis' gland. Uterus overlapping ovary to extending posteriorly to anterior 1/3 anterior testis length, containing 28 (17-30) eggs. Metraterm arising slightly posterior to ventral sucker, weakly muscular, joining distal end of ejaculatory duct at genital pore. Eggs 71 (62-73) long, 34 (26-39) wide. Vitellarium follicular, ventral and dorsal to caeca, with a consistent interruption in distribution at the level of ventral sucker, with or without slight interruption at level of ovary and testes, confluent field in postesticular space; ventral field dense at level of caecal bifurcation and extending to anterior half of ventral sucker, distributed laterally in hindbody anterior to posterior margin of posterior testis, forms confluent field in posttesticular space; dorsal field dense at level of caecal bifurcation and extending to anterior half of ventral sucker, distributed laterally in hindbody anterior to testis, with field extending from testis to nearly the end of the body, median to spanning the body width. Vitellarium with anterior extent at to slightly anterior to intestinal bifurcation, 227 (183-227) from anterior end, representing 18 (16-19)% of BL, posterior extent at 1,224 (965-1,215), representing 98 (95-100)% of BL. Follicles of vitellarium number 144 (113-150), 25-52 (13-68) long, width 27-50 (13-61) wide. Vitelline reservoir median, dorsal to anterior testis, ventral to seminal receptacle. Common vitelline duct joining ootype at level of Mehlis' gland.

Excretory vesicle I-shaped, sac-like, posterior to posterior testis, 62 (50-78) long, representing 5 (5-7)% of BL, 23 (17-38) wide; pore terminal.

Remarks

Plagioporus hendrixi n. sp. can be distinguished from *P. shirleyi, P. chiliticorum, P. crookedensis, P. franksi, P. sinitsini, P. serratus, P. loboides, P. carolini, P. ictaluri, P. fonti, P. limus, P. aliffi, P. boleosomi, P. lepomis, P. serotinus, P. siliculus, P. hypentelii, P. cooperi, P. macrouterinus, P. hageli, P. kolipinskii* and *P. shawi* in the consistent possession of a sinistral, lateral bulge at the level of the genital pore, causing the forebody to be asymmetrical in shape when viewed from a ventral or dorsal aspect. *Plagioporus hendrixi* n. sp. is most similar to *P. shirleyi* in the ratio of body length to width, sucker ratios, possession of oral and ventral suckers that are wider than long, distribution of the vitellarium, extent of the excretory vesicle and parasitism of *H. nigricans*. *Plagioporus hendrixi* n. sp. can be distinguished from *P. shirleyi* in the possession of an internal seminal vesicle representing 74-92% compared with 51-73% of the length of the cirrus sac and in the more posterior position of the ovary (anterior margin at 54-61% of BL as opposed to 50-53% of BL) and testes (anterior testis at 60-68% of BL versus 54-58% of BL; posterior testis at 71-79% of BL versus 65-71% of BL). With the difference in the position of the testes and ovary between the two species, *Plagioporus hendrixi* n. sp. tends to have a shorter postovarian space (31-39% of BL compared with 39-44% of BL) and shorter posttesticular space (7-16% of BL compared with 12-22% of BL). *Plagioporus hendrixi* n. sp. further diverges from *P. shirleyi* in tending to posses a more posteriorly located intestinal bifurcation (19-21% of BL versus 14-19% of BL), a longer forebody (27-31% of BL compared with 25-27% of BL), a longer cirrus sac (19-26% of BL as opposed to 14-19% of BL), larger eggs (62-73 µm versus 58-64 µm long), and fewer vitelline follicles (113-150 compared with 136-179

follicles). Moreover, the vitellarium of *Plagioporus hendrixi* n. sp. tends to have less pronounced interruptions in the hindbody compared with *P. shirleyi*.

Plagioporus shirleyi was not found in *M. erythrurum* nor species of cyprinids, centrarchids, or percids sampled from the type locality (Table 22).

Description of *Plagioporus cf. hypentelii*

Plagioporus cf. hypentelii

Type- and only known-host: *Hypentelium nigricans* (Lesueur), Northern Hog Sucker (Cypriniformes: Catostomidae)

Type-locality: Cosby Creek, Cocke Co., Great Smokey Mountain National Park, Tennessee, U.S.A. (35° 46’ 39.94”N, 83° 12’ 46.75”W)

Site in host: Intestine.

Prevalence: 2 of 2 hosts (100%).

Intensity: 7 per host (average 7).

Type-material: Holotype (USNM XXXXXXX), Paratype (USNM XXXXXXX-X).

Representative DNA sequences: Partial ITS1 and complete ITS2 regions, 5.8S gene, partial (D1–D3) partial (D1–D3) 28S: GenBank accession no. NNXXXXXX, from 2 identical sequences (from separate individual worms).

Description (Figs. 18.11-14)

[Measurements based on 9 gravid wholemounts from *Hypentelium nigricans*] Body white to yellow in life, lanceolate to cylindrical, tapering anteriorly, widest at approximately

2/5 to 1/2 of body length (BL), 693 (626-864) long, 183 (179-214) wide. Oral sucker subterminal, wider than long, 73 (61-79) long, 78 (71-95) wide. Ventral sucker wider than long, 129 (114-154) long, 137 (117-166) wide; width representing 75 (65-78)% of body width. Forebody 247 (212-261), representing 36 (30-36)% of BL. Ratio of oral sucker to ventral sucker width 1:1.8 (1.6-1.8). Prepharynx 10 (9-14) long. Pharynx wider than long, slightly separated from oral sucker, 38 (31-45) long, 45 (40-55) wide. Oesophagus 72 (57-80) long, representing 10 (8-11)% of BL, with or without turn. Intestinal bifurcation 1/2 distance between suckers at 131 (136-160), representing 19 (18-24)% of BL; postcaecal space 53 (47-69) long, representing 8 (7-10)% of BL.

Testes 2, tandem; anterior testis subequal, 103 (85-137) long, 112 (84-139) wide, overlapping caecae ventrally, with anterior margin at 432 (370-549) BL, representing 62 (59-65)% of BL; posterior testis subequal, 116 (86-153) long, 111 (91-129) wide, dorsal to anterior testis, overlapping anterior testis slightly 1/3 of length, with anterior margin at 505 (439-657) BL, representing 73 (70-76)% of BL. Postesticular space 78 (58-94), representing 11 (7-14)% of BL. Cirrus sac clavate, 173 (179-236) long, representing 25 (25-29)% of BL, 50 (36-56) wide, overlapping ventral sucker by 1/2 to all of its length. Vasa efferentia uniting vas deferens at proximal end of cirrus sac. Internal seminal vesicle ovoid, 71 (55-112) long, representing 41 (30-53)% length of cirrus sac, 32 (27-44) at maximum width, communicating with *pars prostatica*. *Pars prostatica* with turn or turns, joining ejaculatory duct in anterior 1/5-2/5 of cirrus sac. Genital pore ventrolateral, sinistral, 156 (122-150) from anterior margin of body, representing 23 (17-23)% of BL.

Ovary ovoid, triangular, or kidney bean-shaped, subequal, 63 (60-91) long, 86 (53-87) wide, dextrally oblique to anterior testis, overlapping anterior testis in posterior 1/3-4/5 of

length, ventrally overlapping dextral caecum, with anterior margin at 412 (365-499) BL, representing 59 (55-58)% of BL. Postovarian space 222 (170-287), representing 32 (25-40)% of BL. Oviduct extending posterodorsally from anterior portion of ovary, joining with canalicular seminal receptacle at midpoint of ovary; seminal receptacle submedian to median, sac-like, dorsal to anterior testis, extending posteriorly to posterior 1/3 of ovary. Laurer's canal opening sinistrally on dorsal surface anterior to ovary. Mehlis' gland median, slightly overlapping to anterior to ovary. Ootype extending anteriorly from seminal receptacle, conspicuous at level of Mehlis' gland. Uterus overlapping ovary to extending posteriorly to anterior 1/3 anterior testis length, containing 6 (4-12) eggs. Metraterm arising slightly posterior to ventral sucker, weakly muscular, joining distal end of ejaculatory duct at genital pore. Eggs 60 (57-64) long, 36 (30-36) wide. Vitellarium follicular, ventral and dorsal to caeca, with slight interruption at level of ventral sucker; ventral field dense at level of intestinal bifurcation and extending to anterior half of ventral sucker, distributed laterally in hindbody, sometimes forming confluent field in postesticular space; dorsal field confluent at level of intestinal bifurcation and extending to anterior half of ventral sucker, distributed laterally in hindbody anterior to testis, forming confluent field in testicular space. Vitellarium with anterior extent at to slightly anterior to intestinal bifurcation, 144 (133-152) from anterior end, representing 21 (16-22)% of BL, posterior extent at 649 (589-828), representing 94 (93-96)% of BL. Follicles of vitellarium number 124 (100-138), 20-45 (19-79) long, width 20-47 (17-60) wide. Vitelline reservoir median, dorsal to anterior testis, ventral to seminal receptacle. Common vitelline duct joining ootype at level of Mehlis' gland.

Excretory vesicle I-shaped, sac-like, posterior to posterior testis to slightly overlapping it, 63 (42-67) long, representing 9 (6-11)% of BL, 24 (13-23) wide; pore terminal.

Remarks

Plagioporus cf. hypentelii can be distinguished from *P. hendrixi*, *P. shirleyi*, *P. chiliticorum*, *P. crookedensis*, *P. franksi*, *P. sinitsini*, *P. serratus*, *P. loboides*, *P. ictaluri*, *P. fonti*, *P. limus*, *P. aliffi*, *P. boleosomi*, *P. lepomis*, *P. serotinus*, *P. siliculus*, *P. cooperi*, *P. macrouterinus*, *P. hageli*, *P. kolipinskii* and *P. shawi* in having the testicular space consistently occupy at least 50% of the length of the hindbody. *Plagioporus cf. hypentelii* can be distinguished from *P. carolini* in lacking an excretory vesicle that reaches the level of the anterior testis and from *P. hypentelii* in having 2 confluent vitelline fields dorsal to the caecae at the level of the intestinal bifurcation and testicular space. The new species is similar to *P. shirleyi* and *P. hendrixi* in having wider than long oral and ventral suckers, a consistent interruption of the vitellarium at the level of the ventral sucker and in common parasitism of *H. nigricans*. *Plagioporus cf. hypentelii* is most similar to *P. hypentelii* in the extent of the testicular space relative to the hindbody, percent body length of the forebody, having ventral vitelline fields distributed laterally from the level of the oesophagus to nearly the end of the body with only a few follicles distributed in the posttesticular space and in common parasitism of *H. nigricans*. *Plagioporus cf. hypentelii* can be further distinguished from *P. hypentelii* in the length of the excretory vesicle despite overlapping body lengths (42-67 µm versus 75-187 µm), possession of an interruption of the vitellarium at the level of the ventral sucker and in having a narrower ventral sucker (117-166 µm compared with 174-240 µm). In addition, the length of the

internal seminal vesicle relative to that of the cirrus sac is shorter in *Plagioporus cf. hypentelii* (30-53% length of cirrus sac versus 63% using Hendrix's (1973) provided measurements of the holotype). Interestingly, measurement Hendrix's (1973)'s illustration of the holotype yielded a seminal vesicle that is 69% of the length of the cirrus sac. The minimum intestinal caecae width relative to body width is wider in *Plagioporus cf. hypentelii* (10-14% of body width compared with 5% of the body width in Hendrix's (1973) illustration of the holotype of *P. hypentelii* and 1% of the body width in the only available paratype of *P. hypentelii*). While Hendrix (1973) fixed the type material of *P. hypentelii* in a hot AFA similar to my specimens that were also fixed in a hot liquid (water), he did so with his specimens under 'slight coverslip pressure,' making comparisons with my material that was fixed without coverslip pressure tenuous. Few specimens of *Plagioporus cf. hypentelii* were available for morphology for this study, so I was unable to fix a series of specimens under slight coverslip pressure to be more comparable to Hendrix's (1973) specimens. Given the difference in fixation methods between *Plagioporus cf. hypentelii* and *P. hypentelii*, I refrain from naming the form from Cosby Creek until material of *P. hypentelii* from its type locality and host fixed without coverslip pressure is available to more thoroughly and definitively distinguish the two species. While I originally intended to redescribe *P. hypentelii*, two separate attempts to sample this species from its type locality in Marsh Creek, Pennsylvania were unsuccessful. I did sample a form of *Plagioporus* from the type locality of *P. hypentelii* from the intestine of *Pimephales promelas* Rafinesque that I consider conspecific with or closely related to *P. crookedensis*, which was originally described from Virginia (Chapter

5) (Fig. 16). Other fish examined from Marsh Creek were negative for *Plagioporus* (Table 23)

Plagioporus cf. hypentelii was not found in *Rhinichthys cataractae* (Valenciennes) (n=4) and *Rhinichthys obtusus* Agassiz (n=11) sampled from Cosby Creek, Tennessee. Examination of specimens prepared by Sherman Hendrix deposited in the Great Smokey Mountains National Museum (USNPS-GRSM- lot VEG) revealed that *C. commersonii* is also parasitized by an intestinal species of *Plagioporus* at Cosby Creek. The conspecificty of this form with *Plagioporus cf. hypentelii* could not be assessed given that the specimens deposited by Hendrix are heavily contracted.

Description of *Plagioporus* sp. A

Plagioporus sp. A.

Only known intermediate host: *Leptoxis carinata (*Brug, 1792) (Cerithioidea: Pleuroceridae)

Locality: intersection of Marsh Creek and US 30, outside of Gettysburg, Pennsylvania (39° 51' 16.89"N, 77° 17' 15.14"W)

Prevalence: 2 of 200 hosts (1%).

Deposited material: Voucher (USNM XXXXXXX-X)

Representative DNA sequences: Complete ITS1 and complete ITS2 regions, 5.8S gene, partial (D1–D3) 28S: GenBank accession no. NNXXXXXX, from 3 identical sequences (from separate individual sporocysts).

Cercaria (Fig. 19.15)

Cercaria colotylomicrocercous (n=6 unless otherwise specified). Body 215-282 long, 52-68 wide; tail 42-64 long, 39-50 wide; total length 259-339. Oral sucker long than wide, 34-43 long, 29-36 wide. Stylet 10-11 long, 4 wide (n=3). Ventral sucker subequal, 33-43 long, 34-44 wide. Pharynx subequal, 14-22 long, 14-24 wide. Excretory vesicle slightly overlapping ventral sucker dorsally, 86-127 long, 240-367 wide.

Sporocyst (Fig. 19.16)

Sporocyst white to yellow in life, elongate cylindrical, with terminal birth pore, 937-1,187 long, 202-341 wide (n=3).

Remarks

While this larval form of *Plagioporus* is described from the same site and host as the original description of the larvae of *P. hypentelii* by Hendrix (1978), I do not consider this form to be conspecific with the larval form described by Hendrix (1978). The length of the sporocyst and cercaria is shorter in *Plagioporus* sp. A as opposed to Hendrix's (1978) larval form (sporocyst 937-1,187 µm versus 2,740-4,260 µm long; body of cercaria 215-282 µm long compared with 515-722 µm long). The cercaria of *Plagioporus* sp. A. also have an excretory bladder that consistently overlaps the ventral sucker, whereas that of the larval form described by Hendrix (1978) extends anteriorly to approximately 3/4 of the distance between posterior margin of the ventral sucker and the end of the body. The fixation methods of the larvae of *Plagioporus* sp. A. and those of *P. hypentelii* by Hendrix (1978) are similar in that there were both heat killed in a hot fluid

(my specimens were heat killed in hot water and immediately transferred to 10% neutral buffered formalin); Hendrix (1978) fixed larval specimens of *P. hypentelii* in hot formalin). My larval specimens were not studied alive apart from observing their movement in a shallow dish. I was unable to observe the esophagus, caecae, flame cells, and penetration glands in *Plagioporus* sp. A (Hendrix [1978] used Neutral red and Nile blue to study living larvae and reported all of these features for *P. hypentelii*).

Molecular Analysis

No intraspecific variation was observed for *Plagioporus shirleyi* n. sp., *Plagioporus hendrixi* n. sp., *Plagioporus cf. hypentelii* and *Plagioporus* sp. A (Table 18). The sequencing reactions of *Plagioporus shirleyi* n. sp. were only successful for the 28S rDNA gene and those for *Plagioporus cf. hendrixi* failed to sequence a portion of the ITS1 rDNA gene. Respective sequence lengths of the complete ITS1 rDNA gene for *Plagioporus hendrixi*, *Plagioporus cf. hypentyelii* and *Plagioporus* sp. A. were 774 bp, 376 bp and 668 bp. For these three species, the lengths of the complete 5.8S and ITS2 rDNA genes were respectively 156 bp, 156 bp and 156 bp and 252 bp, 250 bp and 250 bp. The length of the partial 28S rDNA gene for *Plagioporus shirleyi* n. sp., *Plagioporus hendrixi* n. sp., *Plagioporus cf hypentelii* and *Plagioporus* sp. A were 1,254 bp, 1,319 bp, 1,378 bp, and 1,317 bp, respectively.

In the partial 28S rDNA genes *Plagioporus shirleyi* n. sp. and *Plagioporus hendrixi* n. sp. were most closely related to one another and to *Plagioporus cf. hypentelii*, *Plagioporus* sp. A. and *P. sinitsini*. *Plagioporus cf hypentelii* and *Plagioporus* sp. A. were 100% identical in the 28SrDNA gene, and were found to be most similar to *Plagioporus shirleyi* n. sp., *Plagioporus hendrixi* n. sp., *P. sinitsini*, *P. aliffi* and *P.*

crookedensis. In the complete ITS2 rDNA gene, *Plagioporus hendrixi* n. sp., *Plagioporus cf hypentelii* and *Plagioporus* sp. A. were most closely related to one another (with the latter 2 being 100% identical) and to *P. sinitsini*. In the ITS1 rDNA gene, *Plagioporus hendrixi* n. sp. was closest to *Plagioporus cf. hypentelii*, *Plagioporus* sp. A., and *P. sinitsini*, whereas *Plagioporus cf. hypentelii* and *Plagioporus* sp. A were most closely related to each other (100% identical) and in turn to *P. sinitsini* and *P. crookedensis*. There was no variation in the 5.8S rDNA gene for *Plagioporus hendrixi*, *Plagioporus cf hypentelii*, and *Plagioporus* sp. A (Table 24).

The alignment for the concatenated ITS2 and 28S rDNA tree was 1,723 characters, with 1,025 conserved, 669 variable and 510 parsimony informative sites. The alignment for the 28S rDNA tree was 1,361 characters, with 728 conserved, 619 conserved and 466 parsimony informative sites. The placement of the freshwater plagioporines in both the concatenated and 28S rDNA gene only trees is consistent with previous phylogenies (Bray et al., 2016; Fayton & Andres, 2016; Shedko et al., 2015). The concatenated tree mostly resolved the interelationships between the freshwater plagioporines with higher support and resolution than the 28S rDNA only cladogram. *Plagioporus shawi* was sister with low support to *P. limus*, which in turn was resolved as sister with high support to all other species of *Plagioporus* with sequence data available. The clade sister to *P. limus* was resolved with high support, consisting of a clade formed by *P. loboides*+*P. hageli*+*P. kolipinskii* and another formed by two sister clades, one containing all species known to parasitize catostomids+*Plagioporus* sp. A sister with low support to another composed of 3 intestinal cyprinid parasites + *P.ictaluri* + *P. carolini*. In the catostomid clade, *Plagioporus* cf. *hypentelii* + *Plagioporus* sp. A were sister to

Plagioporus hendrixi n. sp. + *Plagioporus shirleyi* n. sp., and in turn all 4 of these species were sister to *P. sinitsini*, with all relationships resolved with high support. In 28S rDNA only tree, *P. shawi* and in turn *P. limus* were resolved with low and high support, respectively, to all other species of *Plagioporus* with sequence data available, which formed a moderately supported polytomy consisting of 3 clades. These clades included *P. loboides*+*P. hageli*+*P. kolipinskii*, *P. fonti* + *P. boleosomi* + *P. aliffi*, and another clade divided with moderate support into one containing mostly intestinal species from cyprinids + *P. ictaluri*+ *P. carolini* and another that resolved the relationship between *P. sinitsini*, *Plagioporus* sp. A + *Plagioporus* cf *hypentelii*, and *Plagioporus shirleyi* n. sp. + *Plagioporus hendrixi* n. sp. as a polytomy.

Discussion

Morphologically the species of *Plagioporus* from *Hypentelium nigricans* described in this study were very similar to one another, all having wider than long oral and ventral suckers and the vitellarium distributed from the intestinal bifurcation to nearly the end of the body, with an interruption at the level of the ventral sucker. The BI analysis of partial 28S and complete ITS2 rDNA genes confirms that *Plagioporus shirleyi* n. sp., *Plagioporus hendrixi* n. sp. and *Plagioporus cf. hypentelii* are closely related. These three species along with *P. sinitsini* form a monophyletic clade within *Plagioporus*, and these species are notably the only species in the genus included in the analysis that parasitize catostomids. These *Plagioporus* spp. are morphologically distinct in having the vitellarium distributed from the intestinal bifurcation to nearly the end of the body with a distinct median vitelline field dorsal to the testes. The inclusion of *P. sinitsini* in the catostomid clade, a species known to parasitize both catostomids and

cyprinids (Dobrovolny 1939a, Thilakaratne et al., 2007), and the catostomid clade being resolved as sister with moderate support to one mostly composed of species from cyprinids, together suggest a host switching event between cyprinids and catostomids in the eastern Nearctic. *Plagioporus shirleyi* n. sp. and *Plagioporus hendrixi* n. sp. were not found in other hosts sampled from their type localities, including other catostomids (Tables 21 & 22), suggesting that these species may be specific to *H. nigricans*. The apparent host specificity of these new species is consistent with that known for *P. hypentelii*, which matures in *H. nigricans* but not in *C. commersonii* (Hendrix 1973). *Plagioporus cf. hypentelii* was not found in cyprinids from its type locality, but it might occur in *C. commersonii* based on examination of slides from the Great Smokey Mountains Museum deposited by Sherman Hendrix. I recommend that future studies sequence *P. serotinus* to determine its relationship to other species of *Plagioporus* from catostomids, including specimens from its type host (*M. macrolepidotum*), *C. commersonii*, and the form identified as '*P. serotinus'* from *C. carpio*.

The cercaria and sporocyst of *Plagioporus* sp. A. from *L. carinata* from Marsh Creek, Pennsylvania, are morphologically inconsistent with those of *P. hypentelii* described by Hendrix (1978) from the same site and host. I suspect that these 2 larval forms represent two different species of *Plagioporus*. Two species of *Plagioporus* mature in fishes of Marsh Creek: *P. hypentelii* in the intestine of *H. nigricans* (Hendrix, 1973) and *Plagioporus cf. crookedensis* from the intestine of *P. promelas* (this study)(Fig 16). We suspect that the cercaria and sporocyst illustrated by Hendrix (1978) belong to a species of *Plagioporus* other than *P. hypentelii* based on morphological and molecular data. Morphologically, described cercaria of Nearctic *Plagioporus* are smaller in length

than their corresponding adult forms. The relative length of the body of the cercaria to that of the adult in *P. sinitsini, P. lepomis, P. siliculus* and *P. shawi* is respectively 150-300 µm: 691-1,510 µm (Dobrovolny, 1939a), 310-540 µm: 730-1,850 (Dobrovolny, 1939b), 600 µm: 2,500 µm (Sinitsin, 1931) and 296-327 µm: 2,300-4,100 µm (McIntosh, 1939, Schell, 1975). The larval form described by Hendrix (1978) as *P. hypentelii* has a cercarial body 515-722 µm long, whereas adult specimens of *P. hypentelii* range in length from 520-1,162 µm. Thus, it is plausible that the larval form described by Hendrix (1978) has an adult longer than that of *P. hypentelii*. Interestingly, specimens of *Plagioporus cf. crookedensis* from Marsh Creek have a body length over 2,000 µm up to slightly over 3,000 µm; perhaps Hendrix (1978) illustrated the cercaria and sporocyst of this form rather than that of *P. hypentelii* (the metacercaria illustrated by Hendrix [1978] appear to be *P. hypentelii*; this author was likely working with snails infected with multiple species of *Plagioporus*). Molecularly, *Plagioporus* sp. A. was 100% similar to an adult form of *Plagioporus* (*Plagioporus cf. hypentelii*) that is very similar in morphology to *P. hypentelii*, and was nested in a clade of *Plagioporus* spp. that parasitize catostomids, suggesting that *Plagioporus* sp. A is conspecific with or closely related to *P. hypentelii*. I recommend future studies redescribe *P. hypentelii* from its type locality and host using morphological and molecular data to assess its potential conspecificity with *Plagioporus cf. hypentelii* and *Plagioporus* sp. A. Moreover, I recommend that future studies include the complete ITS1 of *Plagioporus cf. hypentelii* in molecular comparisons with *P. hypentelii* to more robustly compare these forms.

Table 18 Species of *Plagioporus* collected from the Nearctic and their respective hosts, collection localities, GenBank accession number (with number of replicates in parenthesis) and deposition information.

Species	Host	Collection Locality and Date		GenBank	NMNH
Plagioporus cf. hypentelii	*Hypentelium nigricans* (Lesueur)	Cosby Creek, TN	7/30/2014	NNXXXXXX (2)	XXXXXX
Plagioporus hendrixi n. sp.	*H. nigricans*	Abrams Creek, TN	7/29/2014	NNXXXXXX (3)	XXXXXX
Plagioporus shirleyi n. sp	*H. nigricans*	Crooked Creek, AR	7/23/2014	NNXXXXXX (3)	XXXXXX
		Poke Bayou, AR	4/23/2016	NA	NA
Plagioporus sp. A	*Leptoxis carinata* (Brug)	Marsh Creek, P.A.	8/20/2013	NNXXXXXX (3)	XXXXXX
Plagioporus cf. crookedensis	*Pimephales promelas* Rafinesque	Marsh Creek, P.A.	8/20/2013	NA	NA
Dimerosaccus oncoyrhynchi (Eguchi, 1913) Shimazu, 1980	*Oncorhynchus masou ishikawae* Jordan & McGregor	Nagara River, Japan	3/11/2012	NNXXXXXX (2)	XXXXXX

Table 19 Sequences obtained from GenBank used for phylogenetic analysis.

Family	Species	Host	GenBank No.	Reference
Brachycladiidae	*Zalophotrema hepaticum* Stunkard & Alvey, 1929	*Zalophus californianus* (Lesson)	AY222255	Olson et al. (2003)
Acanthocolpidae	*Stephanostomum pristis* (Deslongchamps, 1824)	*Phycis phycis* (Linnaeus)	DQ248222	Bray et al. (2005)
Enenteridae	*Enenterum aurem* Linton, 1910	*Kyphosus vaigiensis* (Quoy & Gaimard)	AY222232	Olson et al. (2003)
Lepocreadiidae	*Preptetos caballeroi* Pritchard, 1960	*Naso vlamingii* (Valenciennes)	AY222236	Olson et al. (2003)
Opecoelidae	*Allopodocotyle epinepheli* (Yamaguti, 1942)	*Epinephelus cyanopodus* (Richardson)	KU320598	Bray et al. (2016)
Opecoelidae	*Allopodocotyle margolisi* Gibson, 1995	*Coryphaenoides mediterraneus* (Giglioli)	KU320596	Bray et al. (2016)
Opecoelidae	*Allopodocotyle* sp. A	*Scolopsis bilineata* (Bloch)	KU320599	Bray et al. (2016)
Opecoelidae	*Allopodocotyle* sp. B	*Epinephelus coioides* (Hamilton)	KU320607	Bray et al. (2016)
Opecoelidae	*Anomalotrema koiae* Gibson & Bray, 1984	*Sebastes viviparus* Krøyer	KU320595	Bray et al. (2016)
Opecoelidae	*Bathycreadium brayi* Pérez-del-Olmo, Dallarés, Carrassón & Kostadinova, 2014	*Trachyrincus scabrus* (Rafinesque)	JN085948	Constenla et al. (2011)
Opecoelidae	*Bentholebouria blatta* (Bray & Justine, 2009)	*Pristipomoides argyrogrammicus* (Valenciennes)	KU320608	Bray et al. (2016)
Opecoelidae	*B. blatta*	*Pristipomoides argyrogrammicus*	KU320606	Bray et al. (2016)
Opecoelidae	*Bentholebouria colubrosa* Andres, Pulis & Overstreet 2014	*Pristipomoides aquilonaris* (Goode & Bean)	KJ001207	Andres et al. (2014a)
Opecoelidae	*Biospeedotrema biospeedoi* Bray, Waeschenbach, Dyal, Littlewood & Morand (2014)	*Thermichthys hollisi* (Cohen, Rosenblatt & Moser)	KF733986	Bray et al. (2014)

Opecoelidae	*Biospeedotrema jolliveti* Bray, Waeschenbach, Dyal, Littlewood & Morand (2014)	*Ventichthys biospeedoi* Nielsen, Møller & Segonzac	KF733985	Bray et al. (2014)
Opecoelidae	*Buticulotrema thermichthysi* Bray, Waeschenbach, Dyal, Littlewood & Morand , 2014	*Thermichthys hollisi* (Cohen, Rosenblatt & Moser)	KF733984	Bray et al. (2014)
Opecoelidae	*Cainocreadium labracis* (Dujardin, 1845)	*Gibbula adansonii* (Payraudeau)	JQ694144	Born-Torrijos et al. (2012)
Opecoelidae	*Cainocreadium lintoni* (Siddiqi & Cable, 1960)	*Epinephelus morio* (Valenciennes)	KJ001208	Andres et al. (2014a)
Opecoelidae	*Dimerosaccus oncorhynchi* (Eguchi, 1931)	*Oncorhynchus masou* (Brevoort)	FR870252	Shedko et al. (2015)
Opecoelidae	*Gaevskajatrema halosauropsi* Bray & Campbell, 1996	*Halosauropsis macrochir* (Günther)	AY222207	Olson et al. (2003)
Opecoelidae	*Gaevskajtrema perezi* (Mathias, 1926)	Unidentified fish host	AF184255	Tkach et al. (2001)
Opecoelidae	*Hamacreadium mutabile* Linton, 1910	*Lutjanus griseus* (Linnaeus)	KJ001209	Andres et al. (2014a)
Opecoelidae	*Hamacreadium 'mutabile'*	*Lutjanus fulviflamma* (Forsskål)	KU320601	Bray et al. (2016)
Opecoelidae	*Hamacreadium* sp.	*Lethrinus miniatus* (Forster)	KU320603	Bray et al. (2016)
Opecoelidae	*Helicometra boseli* Nagaty, 1956	*Sargocentron spiniferum* (Forsskål)	KU320600	Bray et al. (2016)
Opecoelidae	*Helicometra epinepheli* Yamaguti, 1934	*Epinephelus fasciatus* (Forsskål)	KU320597	Bray et al. (2016)
Opecoelidae	*Helicometra manteri* Andres, Ray, Pulis, Curran & Overstreet, 2014	*Prionotus alatus* Goode & Bean	KJ701238	Andres et al. (2014b)
Opecoelidae	*H. manteri*	*Bellator egretta* (Goode & Bean)	KJ701239	Andres et al. (2014b)
Opecoelidae	*Maculifer* sp.	*Diodon hystrix* Linnaeus	AY222211	Olson et al. (2003)
Opecoelidae	*Macvicaria bartolii* Antar, Georgieva, Gargouri & Kostadinova, 2015	*Diplodus annularis* (Linnaeus)	KR149464	Antar et al. (2015)
Opecoelidae	*Macvicaria crassigula* (Linton, 1910)	*Calamus bajonado* (Black & Schneider)	KJ701237	Andres et al. (2014b)
Opecoelidae	*Macvicaria dubia* (Stossich, 1905)	*Oblada melanura* (Linnaeus)	KR149469	Antar et al. (2015)

Opecoelidae	*Macvicaria maamouriae* Antar, Georgieva, Gargouri & Kostadinova, 2015	*Sparus aurata* Linnaeus	KR149467	Antar et al. (2015)
Opecoelidae	*Macvicaria macassarensis* (Yamaguti, 1952)	*Lethrinus miniatus* (Forster)	AY222208	Olson et al. (2003)
Opecoelidae	*Macvicaria mormyri* (Stossish, 1885)	Unidentified fish host	AF184256	Tkach et al. (2001)
Opecoelidae	*Macvicaria obovata* (Molin, 1859)	*Cyclope neritea* (Linnaeus)	JQ694147	Born-Torrijos et al. (2012)
Opecoelidae	*M. obovata*	*Gibbula adansonii* (Payraudeau)	JQ694146	Born-Torrijos et al. (2012)
Opecoelidae	*Neolebouria lanceolata* Andres, Pulis & Overstreet, 2014	*Polymixia lowei* (Günther)	KJ001210	Andres et al. (2014a)
Opecoelidae	*Neoplagioporus ayu* (Takahashi, 1928)	*Plecoglossus altivelis altivelis* (Temminck & Schlegel)	XXXXXX	Fayton et al. (2016)
Opecoelidae	*Neoplagioporus elongatus* (Goto & Ozaki, 1930)	*Sarcocheilichthys variegatus microoculus* Mori	XXXXXX	Fayton et al. (2016)
Opecoelidae	*Neoplagioporus zacconis* (Yamaguti, 1934)	*Opsariichthys platypus* (Temminch & Schlegel)	XXXXXX	Fayton et al. (2016)
Opecoelidae	*Opecoeloides fimbriatus* (Linton, 1910)	*Micropogonias undulatus* (Linnaeus)	KJ001211	Andres et al. (2014a)
Opecoelidae	*Opecoeloides furcatus* (Bremser in Rudolphi, 1819)	*Mullus surmuletus* Linnaeus	AF151937	Tkach et al. (2000)
Opecoelidae	*O. furcatus*	*M. surmuletus*	AJ241790	Jousson et al. (1999)
Opecoelidae	*Opistholebes amplicoelus* Nicoll, 1915	*Tetractenos hamiltoni* (Richardson)	AY222210	Olson et al. (2003)
Opecoelidae	*Pacificreadium serrani* (Nagaty & Abdel-Aal, 1962)	*Plectropomus leopardus* (Lacepède)	KU320602	Bray et al. (2016)
Opecoelidae	*Peracreadium idoneum* (Nicoll, 1909)	*Anarhichas lupus* Linnaeus	AY222209	Olson et al. (2003)
Opecoelidae	*Plagioporus aliffi* Fayton, Choudhury, McAllister & Robison, 2017	*Etheostoma blenniodies newmanni* Miller	KX905055	Fayton et al. (2017)

Opecoelidae	*P. boleosomi* (Pearse, 1924)	*Percina maculata* (Girard)	KX553953	Fayton et al. (2016)
Opecoelidae	*P. carolini* Fayton, McAllister & Connior, 201X	*Cottus carolinae* (Gill)	XXXXXX	XXXXXXXXXXX
Opecoelidae	*P. crookedensis* Fayton, 201X	*Clinostomus funduloides* Girard	XXXXXX	XXXXXXXXXXX
Opecoelidae	*P. chiliticorum* (Barger & Esch, 1999)	*Notropis chiliticus* (Cope)	KX553943	Fayton et al. (2016)
Opecoelidae	*P. fonti* Fayton, Choudhury, McAllister & Robsion, 2017	*Percina nigrofasciata* (Agassiz)	KX905054	Fayton et al. (2017)
Opecoelidae	*P. franksi* Fayton, 201X	*Rhinichthys cataractae* (Valenciennes)	XXXXXX	XXXXXXXXXXX
Opecoelidae	*P. hageli* Fayton & Andres, 2016	*Oncorhynchus mykiss* (Walbaum)	KX553950	Fayton et al. (2016)
Opecoelidae	*P. ictaluri* Fayton, Robison, & McAllister, 201X	*Noturus lachneri* Taylor	XXXXXX	XXXXXXXXXXX
Opecoelidae	*P. kolipinskii* Tracey, Choudhury, Cheng & & Ghosh, 2009	*Gasterosteus aculeatus* Linnaeus	KX553952	Fayton et al. (2016)
Opecoelidae	*P. limus* Fayton, Choudhury, McAllister & Robison, 2017	*Etheostoma squamosum* Distler	KX905055	Fayton et al. (2017)
Opecoelidae	*P. loboides* (Curran, Overstreet, & Tkach, 2007)	*Fundulus nottii* (Agassiz)	EF523477	Curran et al. (2007)
Opecoelidae	*P. shawi* (McIntosh, 1939)	*Oncorhynchus tshawytscha* (Walbaum)	KX553951	Fayton et al. (2016)
Opecoelidae	*P. sinitsini* Mueller, 1934	*Notemigonus crysoleucas* (Mitchill)	KX553944	Fayton et al. (2016)
Opecoelidae	*Podocotyloides brevis* Andres & Overstreet, 2013	*Conger esculentus* Poey	KJ001212	Andres et al. (2014a)
Opecoelidae	*Pseudopecoeloides tenuis* Yamaguti, 1940	*Priacanthus hamrur* (Forsskål)	KU320605	Bray et al. (2016)
Opecoelidae	*Pseudopycnadena tendu* Bray & Justine, 2007	*Pseudobalistes fuscus* (Bloch & Schneider)	FJ788506	Bray et al. (2009)
Opecoelidae	*Propycnadenoides philippinensis* Fischthal & Kuntz, 1964	*Gymnocranius grandoculis* (Valenciennes)	KU320604	Bray et al. (2016)

Opecoelidae	*Urorchis acheiloghathi* Yamaguti, 1934	*Tanakia limbata* (Temminck &	KX553945	Fayton et al. (2016)
Opecoelidae	*Urorchis goro* Ozaki, 1927	*Rhinogobius* sp.	KX553946	Fayton et al. (2016)

Table 20 Measurements of *Plagioporus serotinus* Stafford, 1904 from catostomids from new observations of the type material, Stafford (1904), Miller's redescription (1940) and '*Plagioporus serotinus*' identified by Webster (1976) from *Cyprinus carpio* from Montreal, Canada.

	New measurements from type slides (n=1-3)	Stafford, 1904 (n=1)	Redescription of Miller (1940) (n=??)	New measurements from drawings of Miller (1940)	Webster (1976) from *Cyprinus carpio* (n=1-4)
Body Length (BL)	1264-1624 (n=3)	1620	1000-1400*, ≤2200[Ψ]	1229[†], 1619[Ψ]	1378-1683 (n=4)
Body Width (BW)	345-435 (n=3)	430	-	334[†], 438[Ψ]	297-372 (n=4)
BL:BW	1:0.27-0.28 (n=3)	1:0.27	Approx. 1:0.25	1:0.27[†], 1:0.27[Ψ]	1:0.18-.23 (n=4)
Oral Sucker Length, as % BL	128-157, 8-12 (n=3)	138, 9	80-140, NA	109, 0.89[†]; 153, 0.95[Ψ]	114-123, 7-9 (n=4)
Oral Sucker Width, as % BW	115-148, 30-42 (n=3)	138, 32	80-140, NA	107, 32[†]; 131, 30[Ψ]	104-123, 33-37 (n=4)
Ventral Sucker Length, as % BL	177-256, 14-20 (n=3)	231, 14	-	199, 16[†]; 213, 13[Ψ]	200-223, 12-15 (n=4)
Ventral Sucker Width, as % BW	188-271, 54-77 (n=3)	231, 54	-	201, 60[†]; 242, 55[Ψ]	178-201, 54-66 (n=4)
Width of OS:VS	1:1.6-1.9 (n=3)	1:1.5	Approx. 1:2.0	1:1.8[†], 1:1.9[Ψ]	1:1.6-1.9
Pharynx Length, as % BL	66-98, 5-8 (n=3)	-	-	60, 5; 73, 5[Ψ]	90-101, 5-6 (n=2)
Pharynx Width	63-89 (n=3)	-	-	61[†], 80[Ψ]	80-87 (n=2)
Oesophagus Length, as % BL	87, 7; 107, 7 (n=2)	-	-	55[†], 4[†]	88-119, 5-7 (n=2)
Caecal Bifurcation as % BL	20, 22 (n=2)	-	-	20[†]	17-19 (n=3)
Postcaecal Space as % BL	9 (n=1)	-	-	11[†], 11[Ψ]	4-8 (n=4)
Forebody as % BL	29-35 (n=3)	40	-	35[†], 34[Ψ]	31-35 (n=4)
Anterior Testis Length, as % BL	139-182, 11-14 (n=3)	-	-	118, 10[†]; 153, 9[Ψ]	130-171, 9-11 (n=4)
Anterior Testis Width, as % BW	163-216, 45-61 (n=3)	-	-	191, 57[†]; 201, 46[Ψ]	101-160, 33-47 (n=4)
Anterior Testis position as % BL	56, 60 (n=2)	-	-	66[†], 57[Ψ]	61-63 (n=4)
Posterior Testis Length, as % BL	139-212, 11-17 (n=3)	-	-	124, 10[†]; 174, 11[Ψ]	139-186, 10-11 (n=4)
Posterior Testis Width, as % BW	135-214, 39-56 (n=3)	-	-	178, 53[†]; 203, 46[Ψ]	119-177, 40-48 (n=4)
Posterior Testis position as % BL	65, 71 (n=2)	-	-	76[†], 66[Ψ]	70-72 (n=4)
Posttesticular Space as % BL	15-24 (n=3)	-	-	14[†], 23[Ψ]	17-19 (n=4)
Cirrus Sac Length	321 (n=1)	-	-	307[†], 338[Ψ]	313 (n=1)

Cirrus Sac Width	68 (n=1)	-	-	65 †, 60 Ψ	48 (n=1)
Cirrus Length as % BL	20 (n=1)	-	-	21 †, 21 Ψ	19 (n-1)
Cirrus Sac L:W	1:0.21 (n=1)	-	-	1:0.21 †, 1:0.18 Ψ	1:0.15
Seminal Vesicle Length	101 (n=1)	-	-	-	-
Seminal Vesicle Width	48 (n=1)	-	-	-	-
Genital Pore as % Body Length	21, 22 (n=2)	-	-	22 †, 23 Ψ	21 (n=1)
Ovary Length, as % BL	83-110, 7 (n=3)	-	-	85, 7 †; 107, 7 Ψ	99-153, 6-10 (n=4)
Ovary Width, as % BW	89-92, 21-26 (n=3)	-	-	89, 26 †; 107, 24 Ψ	82-120, 27-40 (n=4)
Ovary position as % BL	53, 55 (n=2)	-	-	60 †, 55 Ψ	52-59 (n=4)
Postovarian Space as % BL	39, 42 (n=2)	-	-	33 †, 39 Ψ	34-38 (n=4)
Egg Length	-	-	70-90	84 †	88-96 (n=2)
Egg Width	-	-	50-60	48 †	42-45 (n=2)
Anterior Extent of Vitellarium as % BL	19, 25 (n=2)	-	-	18 †, 18 Ψ	13-16 (n=4)
Posterior extent of Vitellarium, as % BL	91, 95 (n=2)	-	-	96 †, 96 Ψ	94-95 (n=4)
Vitelline follicle length, width	12-44, 8-58 (n=1)	-	-	19-34, 19-32 †; 17-43, 17-36 Ψ	9-59, 13-59; 20-47, 17-45; 29-44, 23-47; 26-51, 19-41

* From *Catostomus commersonii*

Ψ From *Moxostoma valenciennesi*

† Measured from Figure 2 of Miller (1940), presumably from *C. commersonii*

Table 21 Hosts that were negative for *Plagioporus shirleyi* n. sp. from the White River drainage, AR.

Host	Number	Site of Collection	Date
Moxostoma erythrurum (Rafinesque, 1818)	12	Crooked Creek, Marion Co., White River drainage, AR	07/23/2014
Campostoma anomalum (Rafinesque, 1820)	11	Crooked Creek, Marion Co., White River drainage, AR	07/23/2014
Cyprinella galactura (Cope, 1868)	2	Crooked Creek, Marion Co., White River drainage, AR	07/23/2014
Cyprinus carpio Linnaeus, 1758	1	Crooked Creek, Marion Co., White River drainage, AR	07/23/2014
Luxilus pilsburyi (Fowler, 1904)	15	Crooked Creek, Marion Co., White River drainage, AR	07/23/2014
Ameiurus natalis (Lesueur, 1819)	5	Crooked Creek, Marion Co., White River drainage, AR	07/26/2013
Ictalurus punctatus (Rafinesque, 1818)	5	Crooked Creek, Marion Co., White River drainage, AR	07/23/2014
Noturus albater Taylor, 1969	4	Crooked Creek, Marion Co., White River drainage, AR	07/26/2013
Noturus exilis Nelson, 1876	30	Crooked Creek, Marion Co., White River drainage, AR	07/26/2013
Fundulus catenatus (Storer, 1846)	25	Crooked Creek, Marion Co., White River drainage, AR	07/23/2014
Fundulus olivaceus (Storer, 1845)	2	Crooked Creek, Marion Co., White River drainage, AR	07/23/2014
Ambloplites constellatus Cashner & Suttkus, 1977	1	Crooked Creek, Marion Co., White River drainage, AR	07/23/2014
Micropterus dolomieu Lacepède, 1802	10	Crooked Creek, Marion Co., White River drainage, AR	12/18/2013
Micropterus salmoides (Lacepède, 1802)	10	Crooked Creek, Marion Co., White River drainage, AR	07/29/2011
Campostoma oligolepis Hubbs & Greene, 1935	9	Poke Bayou, Independence Co., White River drainage, AR	04/23/2016
Cyprinella galactura (Cope, 1868)	6	Poke Bayou, Independence Co., White River drainage, AR	04/23/2016
Luxilus pilsburyi (Fowler, 1904)	8	Poke Bayou, Independence Co., White River drainage, AR	04/23/2016
Etheostoma caeruleum Storer, 1845	10	Poke Bayou, Independence Co., White River drainage, AR	04/23/2016

Percina caprodes (Rafinesque, 1818)	5	Poke Bayou, Independence Co., White River drainage, AR	04/23/2016
Cottus carolinae (Gill, 1861)	3	Poke Bayou, Independence Co., White River drainage, AR	04/23/2016
Cottus carolinae (Gill, 1861)	3	Poke Bayou, Independence Co., White River drainage, AR	04/25/2013

Table 22 Hosts that were negative for *Plagioporus hendrixi* n. sp. from Abrams Creek, Tennessee

Host	Number	Site of Collection	Date
Moxostoma erythrurum (Rafinesque, 1818)	5	Abrams Creek, Blount Co., Little Tennesse River drainage, Tennessee	07/29/2014
Campostoma anomalum (Rafinesque, 1820)	10	Abrams Creek, Blount Co., Little Tennesse River drainage, Tennessee	07/29/2014
Cyprinella galactura (Cope, 1868)	5	Abrams Creek, Blount Co., Little Tennesse River drainage, Tennessee	07/29/2014
Hybopsis amblops (Rafinesque, 1820)	2	Abrams Creek, Blount Co., Little Tennesse River drainage, Tennessee	07/29/2014
Luxilus coccogenis (Cope, 1868)	4	Abrams Creek, Blount Co., Little Tennesse River drainage, Tennessee	07/29/2014
Nocomis micropogon (Cope, 1865)	10	Abrams Creek, Blount Co., Little Tennesse River drainage, Tennessee	07/29/2014
Notropis leuciodus (Cope, 1868)	10	Abrams Creek, Blount Co., Little Tennesse River drainage, Tennessee	07/29/2014
Notropis telescopis (Cope, 1868)	10	Abrams Creek, Blount Co., Little Tennesse River drainage, Tennessee	07/29/2014
Ambloplites rupestris (Rafinesque, 1817)	3	Abrams Creek, Blount Co., Little Tennesse River drainage, Tennessee	07/29/2014
Micropterus dolomieu Lacepède, 1802	3	Abrams Creek, Blount Co., Little Tennesse River drainage, Tennessee	07/29/2014
Etheostoma chlorobranchium Zorach, 1972	10	Abrams Creek, Blount Co., Little Tennesse River drainage, Tennessee	07/29/2014
Etheostoma tennesseense Powers & Mayden, 2007	2	Abrams Creek, Blount Co., Little Tennesse River drainage, Tennessee	07/29/2014
Etheostoma zonale (Cope, 1868)	8	Abrams Creek, Blount Co., Little Tennesse River drainage, Tennessee	07/29/2014

Table 23 Hosts that were negative for *Plagioporus* from Marsh Creek, Pennsylvania.

Host	Number	Site of Collection	Date
Catostomus commersonii Lacepède, 1803	1	Marsh Creek, Adams Co., Potomac River drainage, Pennsylvania	08/20/2013
Cyprinella analostana Girard, 1859	4	Marsh Creek, Adams Co., Potomac River drainage, Pennsylvania	08/20/2013
Notropis atherinoides Rafinesque, 1818	10	Marsh Creek, Adams Co., Potomac River drainage, Pennsylvania	08/20/2013
Notropis amoenus (Abbott, 1874)	8	Marsh Creek, Adams Co., Potomac River drainage, Pennsylvania	08/20/2013
Semotilus atromaculatus (Mitchill, 1818)	5	Marsh Creek, Adams Co., Potomac River drainage, Pennsylvania	08/20/2013
Pimephales notatus (Rafinesque, 1820)	10	Marsh Creek, Adams Co., Potomac River drainage, Pennsylvania	08/20/2013
Etheostoma sp.	4	Marsh Creek, Adams Co., Potomac River drainage, Pennsylvania	08/20/2013
Fundulus diaphanous (Lesueur, 1817)	3	Marsh Creek, Adams Co., Potomac River drainage, Pennsylvania	08/20/2013
Ambloplites rupestris (Rafinesque, 1817)	4	Marsh Creek, Adams Co., Potomac River drainage, Pennsylvania	08/20/2013
Lepomis cyanellus Rafinesque, 1819	3	Marsh Creek, Adams Co., Potomac River drainage, Pennsylvania	08/20/2013
Lepomis gibbosus (Linnaeus, 1758)	3	Marsh Creek, Adams Co., Potomac River drainage, Pennsylvania	08/20/2013
Micropterus dolomieu Lacepède, 1802	2	Marsh Creek, Adams Co., Potomac River drainage, Pennsylvania	08/20/2013

Table 24 Pairwise comparisons of percent nucleotide similarity and the number of base pair differences (in parentheses) for the 28S, ITS2, ITS1 and 5.8S of *Plagioporus shirleyi* n. sp., *Plagioporus hendrixi* n. sp., *Plagioporus cf. hypenteii*, *Plagioporus* sp. A. and congeners.

		Plagioporus hendrixi n. sp.	*Plagioporus cf. hypentelii*	*Plagioporus* sp. A	*Plagioporus crookedensis*	*Plagioporus franksi*	*Plagioporus fonti*	*Plagioporus limus*	*Plagioporus aliffi*	*Plagioporus boleosomi*	*Plagioporus chilliticorum*	*Plagioporus hageli*	*Plagioporus kolipinskii*	*Plagioporus sinitsini*	*Plagioporus shawi*	*Plagioporus loboides*	*Plagioporus carolini*	*Plagioporus ictaluri*
28S	*Plagioporus shirleyi* n. sp.	99.8 (3)	98.2 (22)	98.2 (22)	97.6 (30)	97.6 (30)	96.9 (38)	97.1 (35)	97.6 (30)	96.8 (39)	97.5 (31)	97.1 (35)	96.2 (46)	98.0 (25)	95.7 (52)	97.4 (30)	96.6 (42)	96.4 (44)
28S	*Plagioporus hendrixi* n. sp.	-	98.3 (23)	98.3 (23)	97.8 (29)	97.7 (30)	97.0 (40)	97.3 (36)	97.5 (33)	96.9 (41)	97.6 (31)	97.2 (37)	96.4 (48)	98.0 (26)	95.9 (53)	97.3 (31)	96.7 (44)	96.4 (47)
28S	*Plagioporus cf. hypentelii*	-	-	100.0 (0)	98.3 (23)	97.8 (30)	97.8 (29)	97.8 (29)	98.7 (18)	97.7 (30)	97.7 (31)	97.8 (29)	96.4 (48)	98.4 (21)	96.4 (47)	98.0 (23)	96.9 (41)	96.7 (44)
28S	*Plagioporus* sp. A.	-	-	-	98.3 (23)	97.7 (30)	97.8 (29)	97.8 (29)	98.6 (18)	97.7 (30)	97.6 (31)	97.8 (29)	96.4 (48)	98.4 (21)	96.4 (47)	98.0 (23)	96.9 (41)	96.7 (44)
ITS2	*Plagioporus shirleyi* n. sp.	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
ITS2	*Plagioporus hendrixi* n. sp.	-	98.0 (5)	98.0 (5)	96.4 (9)	94.4 (14)	95.2 (12)	94.0 (15)	94.8 (13)	94.8 (13)	94.0 (15)	95.6 (11)	93.5 (16)	98.0 (5)	87.1 (31)	NA	94.0 (15)	93.6 (16)
ITS2	*Plagioporus cf. hypentelii*	-	-	100.0	97.6 (6)	95.6 (11)	96.4 (9)	95.2 (12)	96.8 (8)	96.8 (8)	95.2 (12)	96.8 (8)	95.6 (11)	99.2 (2)	88.3 (28)	NA	96.0 (10)	95.6 (11)
ITS2	*Plagioporus* sp. A.	-	-	-	97.6 (6)	95.6 (11)	96.4 (9)	95.2 (12)	96.8 (8)	96.8 (8)	95.2 (12)	96.8 (8)	95.6 (11)	99.2 (2)	88.3 (28)	NA	96.0 (10)	95.6 (11)
ITS1	*Plagioporus shirleyi* n. sp.	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
ITS1	*Plagioporus hendrixi* n. sp.	-	96.8 (12)	92.6 (48)	91.0 (58)	85.7 (92)	86.4 (87)	86.9 (81)	88.1 (76)	87.1 (84)	86.0 (90)	78.9 (156)	75.3 (165)	91.8 (52)	81.3 (93)	NA	87.1 (82)	87.0 (82)
ITS1	*Plagioporus cf. hypentelii*	-	-	100.0 (0)	97.1 (11)	94.9 (19)	92.8 (27)	95.2 (18)	93.6 (24)	92.6 (28)	95.5 (17)	93.6 (24)	92.0 (30)	98.1 (7)	88.3 (44)	NA	94.2 (22)	94.2 (22)

ITS1	*Plagioporus* sp. A.	-	-	-	91.5 (55)	86.3 (89)	87.4 (81)	87.3 (79)	88.7 (73)	87.7 (80)	86.6 (87)	84.4 (101)	77.8 (142)	93.2 (44)	82.0 (89)	NA	87.6 (79)	87.7 (78)
5.8S	*Plagioporus shirleyi* n. sp.	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
5.8S	*Plagioporus hendrixi* n. sp.	-	100.0 (0)	100.0 (0)	98.7 (2)	99.4 (1)	98.7 (2)	98.7 (2)	98.7 (2)	98.7 (2)	99.4 (1)	98.7 (2)	99.4 (1)	99.4 (1)	100.0 (0)	NA	98.7 (2)	98.7 (2)
5.8S	*Plagioporus cf. hypentelii*	-	-	100.0 (0)	98.7 (2)	99.4 (1)	98.7 (2)	98.7 (2)	98.7 (2)	98.7 (2)	99.4 (1)	98.7 (2)	99.4 (1)	99.4 (1)	100.0 (0)	NA	98.7 (2)	98.7 (2)
5.8S	*Plagioporus* sp. *A.*	-	-	-	98.7 (2)	99.4 (1)	98.7 (2)	98.7 (2)	98.7 (2)	98.7 (2)	99.4 (1)	98.7 (2)	99.4 (1)	99.4 (1)	100.0 (0)	NA	98.7 (2)	98.7 (2)

Figure 15. Plagioporus serotinus from the intestine of *Moxostoma macrolepidotum*. 1, Ventral view; 2, Dorsal view showing dorsal vitelline fields; *Scale bars* 100 μm

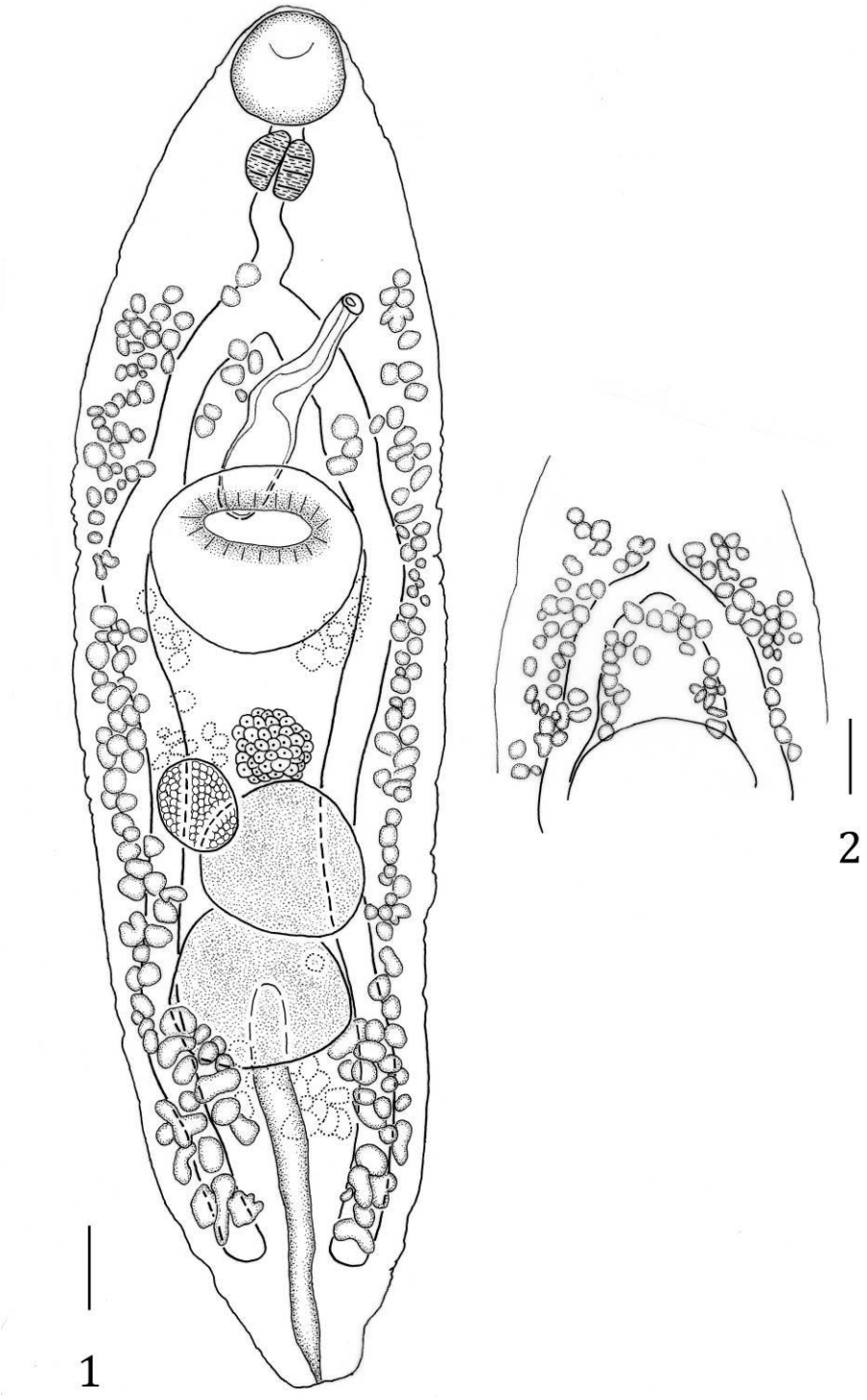


Figure 16. *Plagioporus shirleyi* n. sp. from the intestine of *Hypentelium nigricans*. 3. Ventral view showing ventral vitelline fields and dorsal field in testicular space; 4, Dorsal view showing dorsal vitelline fields; 5, Terminal genitalia, ventral view; 6, Female complex, dorsal view. *Scale bars* 100 μm for 3-4 and 50 μm for 5-6.

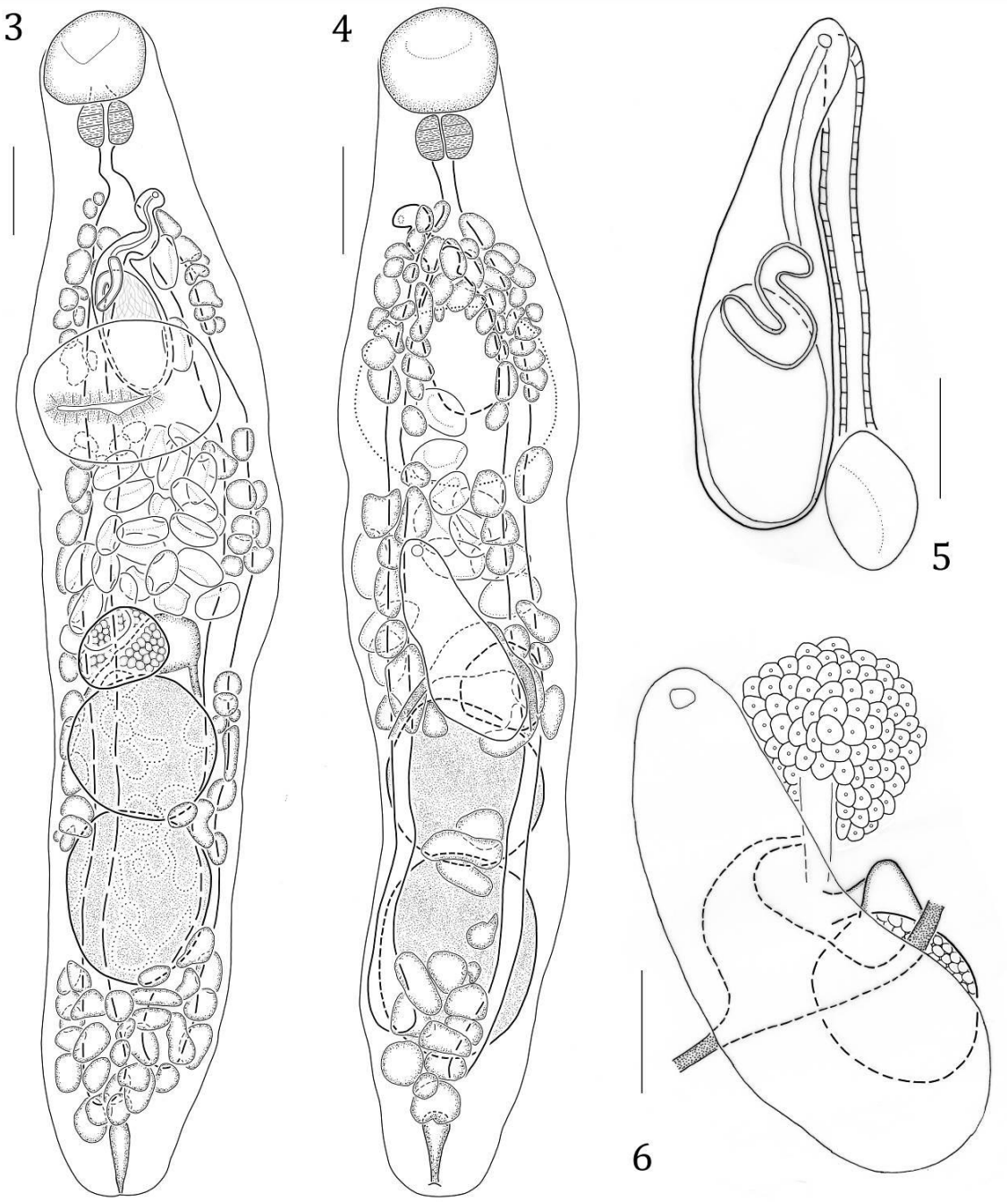


Figure 17. *Plagioporus hendrixi* n. sp. from the intestine of *Hypentelium nigricans*. 7. Ventral view showing ventral vitelline fields and dorsal field in testicular space and level of ovary; 8, Dorsal view showing dorsal vitelline fields; 9, Terminal genitalia, ventral view; 10, Female complex, dorsal view. *Scale bars:* 100 µm for 7-8 and 50 µm for 9-10.

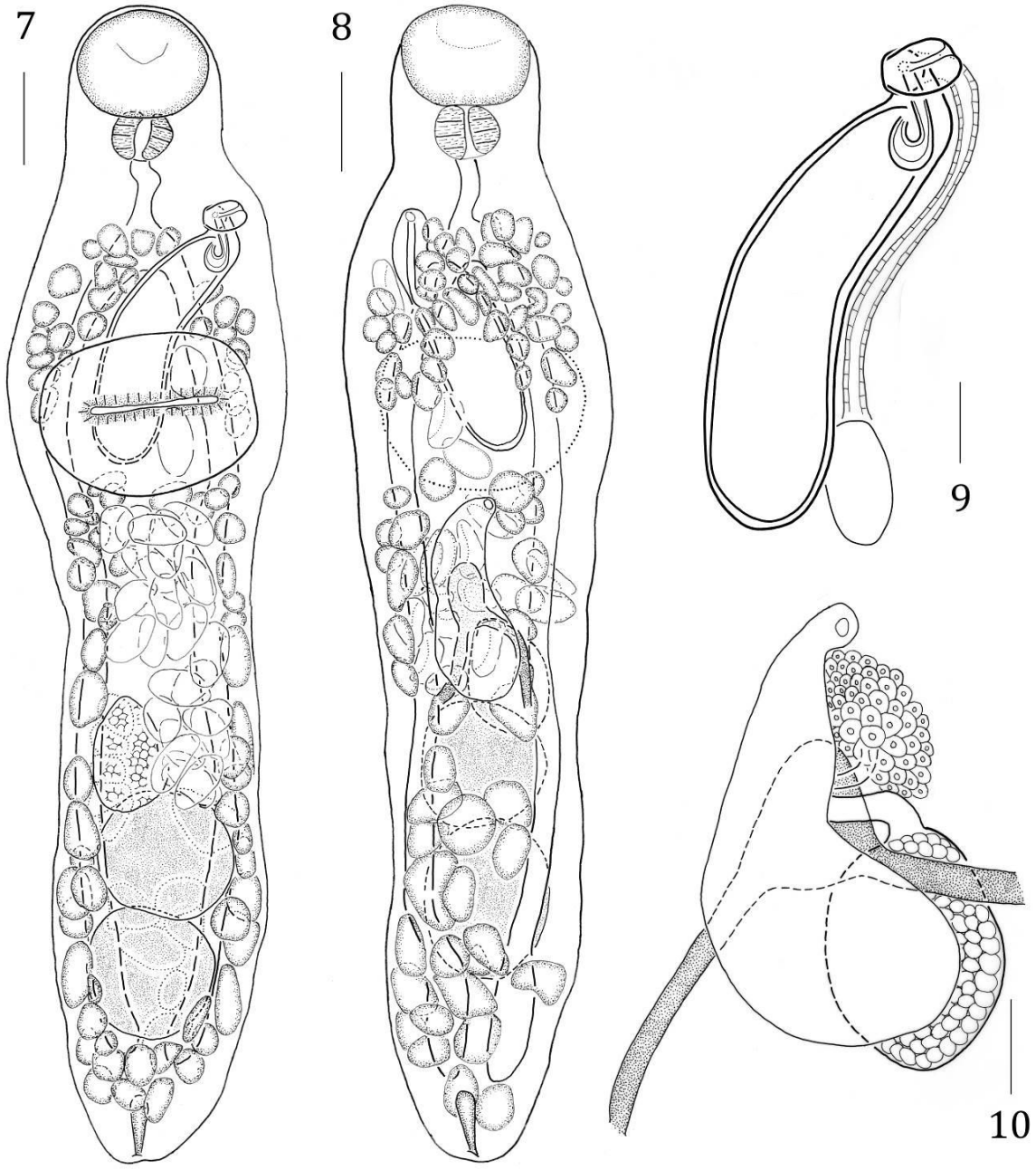


Figure 18. *Plagioporus* cf *hypentelii* from the intestine of *Hypentelium nigricans*. 11. Ventral view showing ventral and dorsal vitelline fields; 12, Dorsal view showing dorsal vitelline fields; 13, Terminal genitalia, ventral view; 14, Female complex, dorsal view. *Scale bars:* 100 μm for 11-12 and 50 μm for 13-14

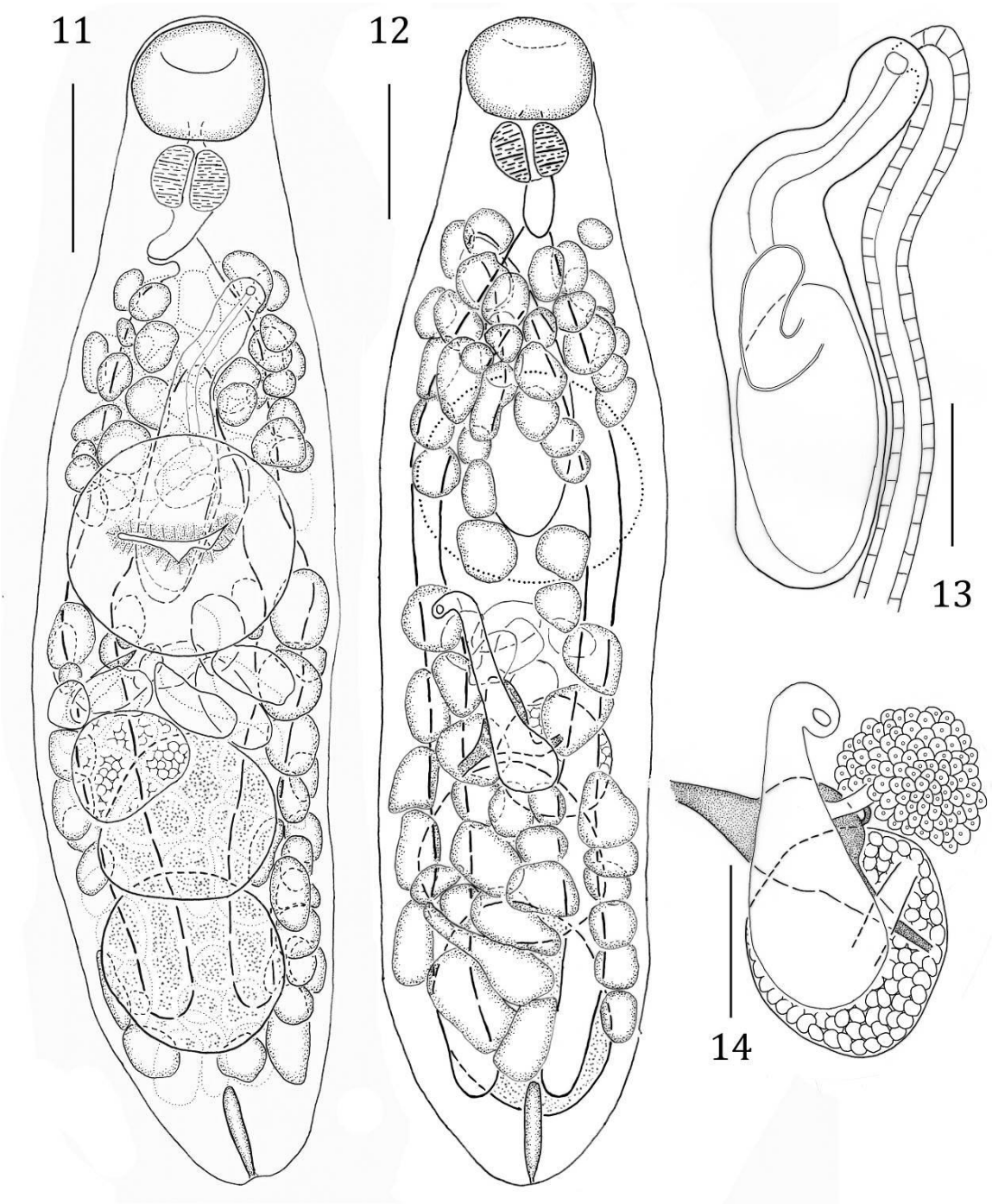


Figure 19. Plagioporus sp. A from *Leptoxis carinata* from Marsh Creek Pennsylvania. 15. Sporocyst. 16. Cercaria. *Scale bars:* 100 μm

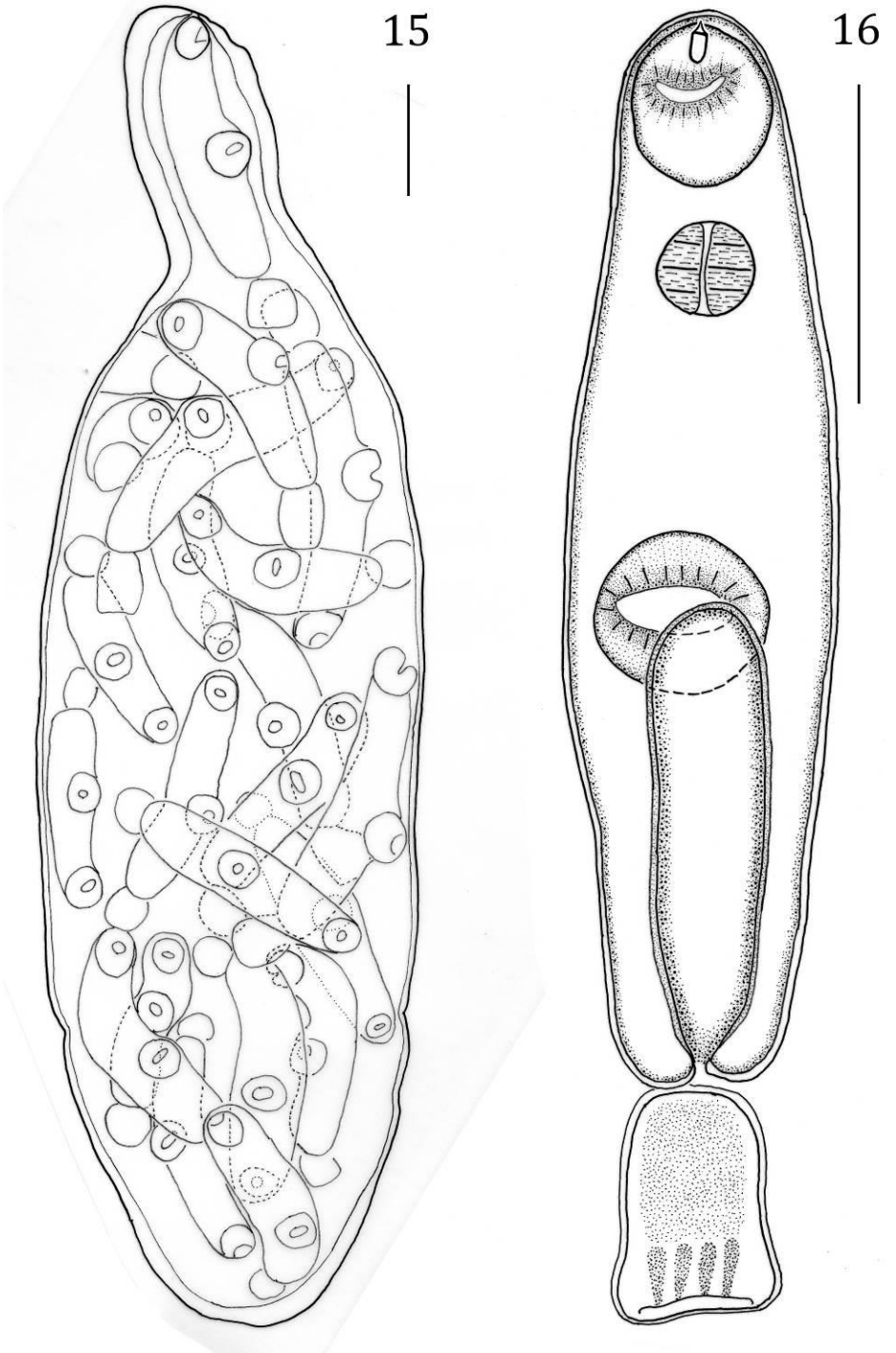
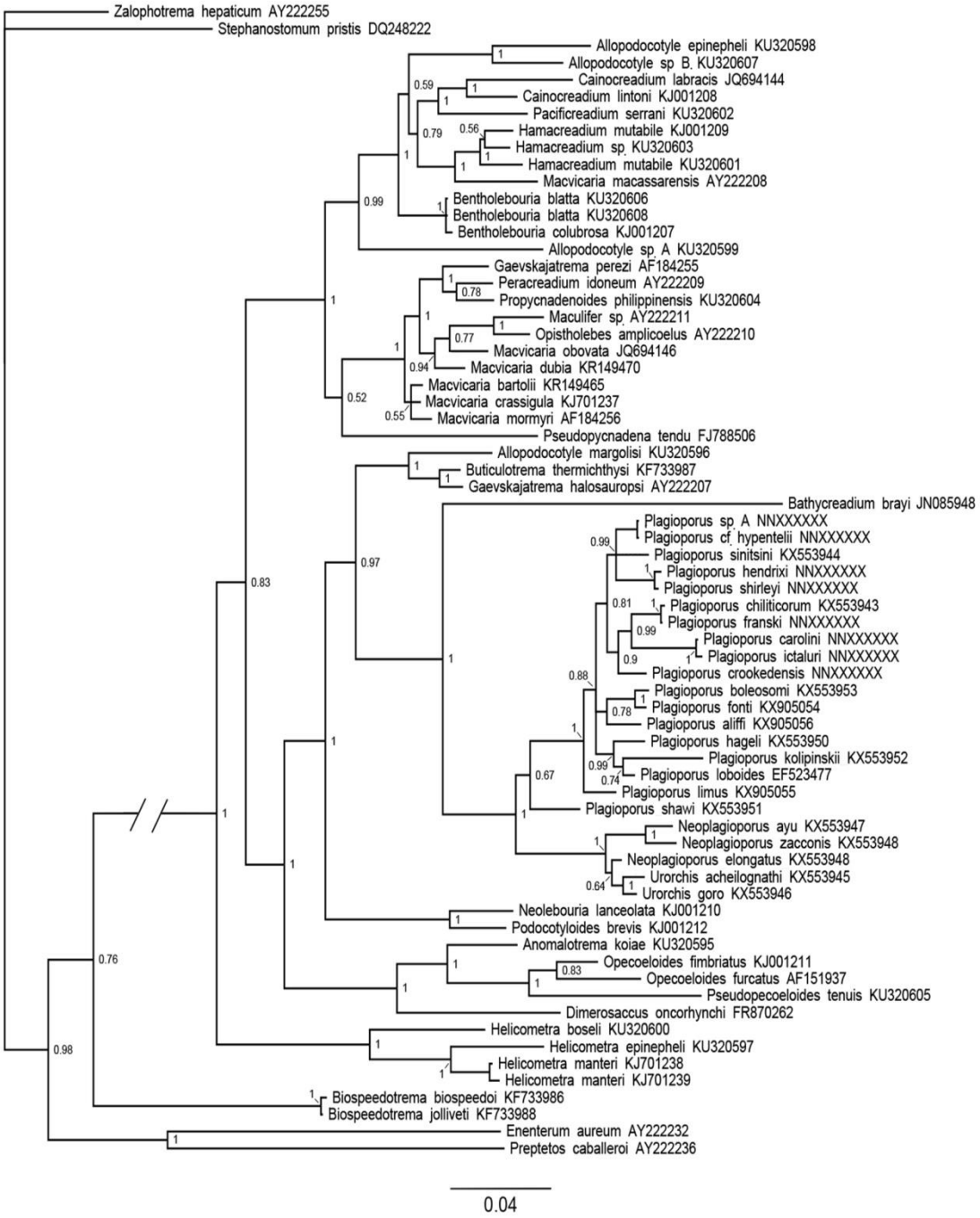


Figure 20. Phylogenetic relationships of opecoelids resulting from Bayesian inference analysis of partial 28S rDNA sequences (GTR + I + Γ) (5,000,000 generations and a sample frequency of 1,000) (Length of truncated branch=0.13).



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CHAPTER VII - Conclusion

Hypothesis 1: Freshwater plagioporines, including *Plagioporus* Stafford, 1904, represent monophyletic groups

Previous to this research, only a single species of plagioporine parasitizing a freshwater host had been included in a molecular phylogeny of the opecoelids, *Plagiocirrus loboides* (Curran, Overstreet & Tkach) Fayton & Andres, 2016 (Andres et al., 2014; Bray et at., 2014, 2016; Fayton et al., 2016; Olson et al., 2003; Shedko et al., 2015). For this dissertation, I obtained sequences from 5 previously described species, 10 new species and a new form of *Plagioporus* from North America, 3 species of *Neoplagioporus* from Japan, including the type species, as well as 2 species of *Urorchis*, including the type species, also from Japan. While this collection of sequences only represents 3 of the 17 genera and 21 of the approximately 85 species of freshwater plagioporines, it constitutes the largest assemblage of sequences of freshwater plagioporines available thus far. For *Plagioporus*, 16 of the 23 accepted Nearctic species in the genus were sequenced. Phylogenetic trees using BI analysis of the 28S rDNA gene and also the ITS2 region concatenated with the 28S rDNA gene revealed that the freshwater plagioporines form a monophyletic group within the Opecoelidae. Consistent with previous phylogenies, which all examined rDNA (Andres et al., 2014; Bray et at., 2014, 2016; Fayton et al., 2016; Olson et al., 2003; Shedko et al., 2015) the freshwater plagioporines were found to be most closely related to the deep water marine plagioporines and the only opecoelinine with sequence data available, *Buticulotrema thermichthysi*, which is also a deep water marine species. Thus, given that the opecoelids are a largely marine group and the plagioporines from freshwater hosts form a derived

clade within the opecoelids, the clade consisting of the freshwater plagioporines constitutes a single, monophyletic radiation into freshwater fish hosts from ancestors with marine/estuarine hosts. This represents one of at least 2 radiations of marine/estuarine opecoelids into freshwater fish hosts, with the other occurring in the opecoeline clade. Freshwater plagioporines from the Nearctic were sister to those from the Palearctic with high support. The monophyly of *Plagioporus* is a more complicated matter. While the freshwater plagioporines from the Nearctic form a monophyletic group, *Plagiocirrus loboides* nested within the clade containing all other species of *Plagioporus*, making *Plagioporus* a paraphyletic assemblage. Morphological analysis revealed that the characters used to distinguish *Plagiocirrus* from *Plagioporus*, especially a reduced vitellarium, are represented at least to an extent in *Plagioporus*. Given this finding, the nesting of *P. loboides* within *Plagioporus*, and that *Plagioporus* has priority over *Plagiocirrus*, I transferred *P. loboides* to *Plagioporus*, making *Plagioporus* a monophyletic group, and amended *Plagioporus* to accommodate a uterus that may extend to the posterior end of the body and a reduced vitelline field. I additionally predict that *Plagiocirrus* will be subsumed under *Plagioporus* once sequences of the type species of *Plagiocirrus* are available. The morphological variation of *Plagioporus* is further expanded in Chapter 3, in which I amend *Plagioporus* to accommodate 2 genetically and morphologically new species of *Plagioporus* from Arkansas. The excretory vesicle of these new species, *P. carolini* and *P. ictaluri*, extends anteriorly at least to the level of the anterior testis, a character state that currently violates the diagnosis of *Plagioporus* (Gibson & Bray [1982] restricted *Plagioporus* to freshwater forms with an excretory vesicle extending anteriorly to at most the level of the posterior testis). Accordingly, I

further amend *Plagioporus* to accommodate an excretory bladder reaching the level of the anterior testis and ovary. This amendment is supported by the morphology one of the new species of *Plagioporus* described in Chapter 4, *P. limus*, which has an excretory vesicle that can nearly reach the level of the ovary. Such a long excretory vesicle may not be a novel character for *Plagioporus*; it is seen in other freshwater plagioporines as well, including the type species of *Plagiocirrus* and members of *Urorchis* and *Neoplagioporus*.

The monophyly of the Palearctic freshwater plagioporine genera is also complicated. While all 5 species of *Neoplagioporus* and *Urorchis* from Japan formed a highly supported monophyletic group, *Neoplagioporus elongatus* nested within a clade containing 2 species of *Urorchis* and not with its 2 congeners included in the analysis. The characters that have been used to distinguish *Urorchis* from *Neoplagioporus* include a uterus that reaches the end of the body and the embryonation of the eggs. I was unable to discern a difference in the embryonation of the eggs between specimens of *Urorchis* and *Neoplagioporus* available to me. Moreover, whereas the uterus of *Neoplagioporus* is usually pretesticular, but it can extend posteriorly to the middle of the posterior testis a very short distance from the end of the body in the type-species, *N. zacconis* (Shimazu, 1990a). A reduced vitellarium has also been used to distinguish *Urorchis* from *Neoplagioporus*, although morphological examination revealed that the proportion of the body length occupied by the vitellarium can overlap in these two genera (Shimazu, 1990a, b), as is the case with *Plagioporus* and *Plagiocirrus*. Given that the posterior extent of the uterus and a reduced vitellarium may not be useful characters in distinguishing opecoelid genera (e.g. *Plagiocirrus*) and the unresolved placement of *N. elongatus*, I could be justified in reducing *Neoplagioporus* to a junior synonym of

Urorchis (the type species of both genera are represented in the phylogeny). However, I chose to await molecular data for additional species of *Urorchis*, *Neoplagioporus* and other freshwater plagioporines from the Palearctic that might clarify the observed phylogenetic relationships. It is likely that *Urorchis* will have to be amended to accommodate *N. elongatus* and most probably all other species of *Neoplagioporus* as well.

The Opecoelidae poses a challenge to taxonomists in that it is not only the largest family of digeneans but it is also rife with homoplasy (Bray et al., 2016). Many of the characters used to distinguish opecoelid genera are weak and often grade into one another. The freshwater plagioporines are no exception to this trend. The 17 freshwater genera are in many cases poorly distinguished from one another, particularly for the 14 genera with blindly ending caecae. *Plagioporus*, for example, is not clearly morphologically differentiated from the Japanese genera *Neoplagioporus* and *Urorchis* collectively; the only character that that can be used to definitely distinguish these genera is the possession of a contiguously bipartite seminal vesicle in the Japanese worms. The seminal vesicle of several species of *Plagioporus* from the Nearctic is bipartite, but not contiguously bipartite, with chambers of the vesicle separated by a distinct duct. However, we suspect that this distinguishing character will become problematic given the contiguously bipartite seminal vesicle of a new species that has yet to be described from Wisconsin and *Pseudurorchis catostomi* Schell, 1974 described from *C. macrocheilus* from the Clearwater River in Idaho, USA, a species we suspect belongs in *Plagioporus*. Schell (1974) likely created an unnatural group in assigning the latter species to *Pseudurorchis* Yamaguti, 1971, which at the time contained two species from freshwater

fish in Israel (Schell, 1974). Apart from *Pseudurorchis*, the only other freshwater plagioporine genus with a distribution that spans the Holarctic is *Plagioporus*, which also may prove to be an unnatural grouping. We suspect that *Plagioporus* will prove to be a Nearctic genus given that it was erected for an opecoelid from a Nearctic freshwater fish and is phylogenetically distinct from Palaearctic freshwater plagioporines from which it is not clearly distinguished. To test this hypothesis, species currently assigned to *Plagioporus* distributed in the Palearctic need to be included in molecular phylogenies of freshwater plagioporines along with the other genera parasitizing freshwater fish in this ecozone. While this study has elucidated the evolution of the freshwater plagioporines, there is much work to be done, especially considering the small fraction of species and genera with sequence data available compared to those that have been described. One of the major finding of this dissertation is the increased morphological variation realized for *Plagioporus*. We suspect that as additional species are described and sequenced from freshwater hosts, the number of genera of freshwater plagioporines will ultimately decrease as various genera are consolidated. In the Nearctic, 5 of the 7 genera of freshwater plagioporines are either monotypic (*Multivitellina*, *Pseudopodocotyle* and *Nezpercella*) or only have one representative in Nearctic freshwater fish, possibly representing unnatural groupings (*Allopodocotyle virens* and *P. catostomi*). It is not unlikely that some or all of these genera will be subsumed under *Plagioporus* once sequence data becomes available (*Plagioporus* has priority over all of them).

Hypotheses 2 and 3: *Plagioporus* has radiated across many families of freshwater/anadromous fish. Intestinal *Plagioporus* of the Nearctic have radiated

within a range of fish families with freshwater/anadromous members, particularly for the percids, cyprinids and catostomids

Previous to this work, Tracey et al. (2009) recognized 13 nominal species of *Plagioporus* from the Nearctic, with 11 intestinal species parasitising cyprinid, catostomid, percid, salmonid, gasterosteid, anguillid and centrarchid hosts and two gall bladder species from cyprinid, catostomid and hiodontid hosts. Sequences (28S rDNA and ITS2+28S rDNA) of new and previously described species of *Plagioporus* collected from the Nearctic from cyprinid, catostomid, percid, salmonid, gasterosteid, fundulid, ictalurid, and cottid definitive hosts formed a monophyletic group within the opecoelids, supporting Tracey et al.'s (2009) observation that the diversification of *Plagioporus* has involved considerable host shifting, leading to a diverse host assemblage. This work represents this first to describe a species of *Plagioporus* from an ictalurid and the first to describe a species from a cottid east of the Rocky Mountains. In addition, with the the transfer of 'Plagiocirrus loboides' to *Plagioporus*, this work represents the first report of a member of *Plagioporus* parasitizing a fundulid. With 23 species, *Plagioporus* is now the most diverse digenean genus of fish in the Nearctic and one of the most successful in terms of its radiation across fish host families. Of the other digenean genera of freshwater fish in the Nearctic, only 2 rival *Plagioporus* with respect to the diversity of definitive host families: *Phyllodistomum* Braun, 1899 (21 species across 11 fish host families) and *Crepidostomum* Braun, 1900(14 species across 16 fish host families) (Hoffman, 1999; Choudhury et al., 2016).

With respect to radiations within fish families, it seems that *Plagioporus* has radiated within salmonids in the Pacific Northwest; *P. hageli*, a new intestinal species

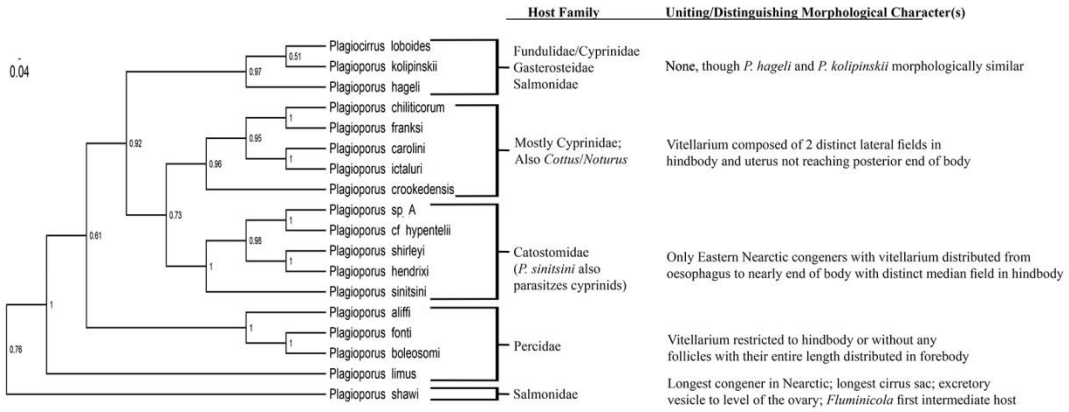
described from *Oncorhynchus mykiss* from California, was very morphologically similar to two forms reported by Haderlie (1953) from the same host, but different drainages, in California (*P. hageli* occurs and the Sacramento River drainage and Haderlie's (1953) forms were reported from the Klamath and Truckee River drainages). Unfortunately, collection of these two forms reported by Haderlie (1953) was unsuccessful; without sequence data, the monophyly of this apparent radiation cannot be tested. At the very least, it is apparent that the diversification of *Plagioporus* in salmonids is complex; the lack of a close relationship between *P. shawi* and *P. hageli* suggests 2 independent radiations into salmonids in the Pacific Northwest.

Monophyletic radiations of intestinal *Plagioporus* have occurred within the percids, cyprinids and catostomids, and this is supported by both morphological and molecular data (Fig. 21). For the percids, I described 3 new species of *Plagioporus* from darters from Florida and Arkansas and redescribed the only congener previously described from this host family, *P. boleosomi* from *Etheostoma nigrum* Rafinesque (type host) and *Percina* spp. from Wisconsin. Morphological comparisons revealed that species of *Plagioporus* from darters are morphologically unqiue from congeners in having the vitellarium absent in the forebody or without follicles with their entire length distributed in the forebody and in possession of a confluent to nearly confluent vitelline field in the posttesticular space. Bayesian Inferences (BI) analysis of rDNA sequences (28S and also 28S concatenated with ITS2) resolved three of these species, *P. boleosomi*, *P. aliffi* and *P. fonti* as a monophyletic clade within *Plagioporus* with highly supported interrelationships. As to whether the other species of *Plagioporus* from a darter, *P. limus*, is part of this radiation or represents a second radiation into darter hosts is unclear. It is

possible that the resolution of *P. limus* on a basal clade of *Plagioporus* as opposed to a more internal node like its congeners from darters is an artifact of insufficient sequence data on forms from darters. Based on my morphological comparisons of *P. aliffi* to a form described by Aliff (1973), undocumented diversity of *Plagioporus* persists in darter hosts. Sequencing of this form, others that are new, and also *P. lepomis*, which shares several morphological features with the forms from darters, will hopefully clarify the interelaionships between the species of *Plagioporus* parasitizing this host family. With respect to the radiation of *Plagioporus* within cyprinid hosts, I redescribed *Plagioporus chiliticorum* from its type host, *Notropis chiliticus* (Cope), and locality in North Carolina and described 2 new species from cyprinids, including *P. crookedensis* from *Clinostomus funduloides* Girard from Virginia and *P. franksi* from *Rhinichthys* spp. from Tennessee. Morphologically, these three forms from cyprinids are unqiue from Nearctic congeners in possession of a bipartite seminal vesicle and in having the vitellarium in two distinct vitelline fields in the hindbody. BI analysis of the 28S rDNA sequences resolved the species from cyprinids and two closely related species from a cottid and an ictalurid, *P. carolini* and *P. icatluri*, as a monphyletic clade within *Plagioporus*, and these 5 species are notably the only of those with sequence data available that have the vitellarium restricted to two distinct fields in the hindbody and without a uterus that extends to the end of the body. The nesting of *P. carolini* and *P. ictaluri* in a clade composed of congeners with cyprinid hosts may suggest a host switching event between cyprinids and cottids/ictalurids at some point in the evolutionary history of *Plagioporus*. Lastly, with respect to a radiation within the catostomids, I redescribed the type species of *Plagioporus*, *P. serotinus*, from the type material and other specimens deposited in the

Canadian Museum of Nature. Two separate attempts to collect *P. serotinus* from catostomids from Canada were unfortunately unsuccessful. However, I did describe 2 new species, *P. shirleyi* and *P. hendrixi*, and a new form, *Plagioporus* cf *hypentelii*, all from the same catostomid host, *Hypentelium nigricans*, from Tennessee and Arkansas. Additionally, I discovered a larval form in a pleurocerid snail in Pennsylvania at the type locality of the only previously described species of *Plagioporus* from *H. nigricans*, *P. hypentelii*. *Plagioporus* cf *hypentelii* collected from Tennessee may be a new species of *Plagioporus* based on morphological differences from its closest congener, *P. hypentelii*. However, as these differences could be an artifact of the contracted nature of the type material of *P. hypentelii* and fresh material of *P. hypentelii* was not available, I refrained from calling this form definitively new. Interestingly, *Plagioporus* cf *hypentelii* is geneticially identical in the partial ITS1, complete ITS2, and partial 28S to the larval form of *Plagioporus* from Pennsylvania that I suspect is conspecific with *P. hypentelii*. BI analyses of the concatenated and 28S rDNA only allignments both resolved a monophyletic clade within *Plagioporus* composed of the forms from *H nigricans*, the larval form of *Plagioporus* from Pennsylvania that is probably *P. hypentelii*, and also *P. sinitsini*, which is known to parasitize the gall bladder of both catostomids and cyprinids. The members of this catostomid clade are unqiue in being the only Eastern congeners with the vitellarium distributed from the level of the esophagus to nearly the end of the body with a dorsal, median vitelline field in the hindbody. Both concatenated and 28S rDNA only BI analyses resolved the clade of catostomid species of *Plagioporus* as sister to that containing mostly cyprinid congeners. Host switching between the cyprinids and catostomids is likely facilitated by both host families being cypriniformes.

Figure 21. Plagioporus clade from 28S+ITS-2 rDNA cladogram illustrating how morphology and host family influence phylogenetic relationships.



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